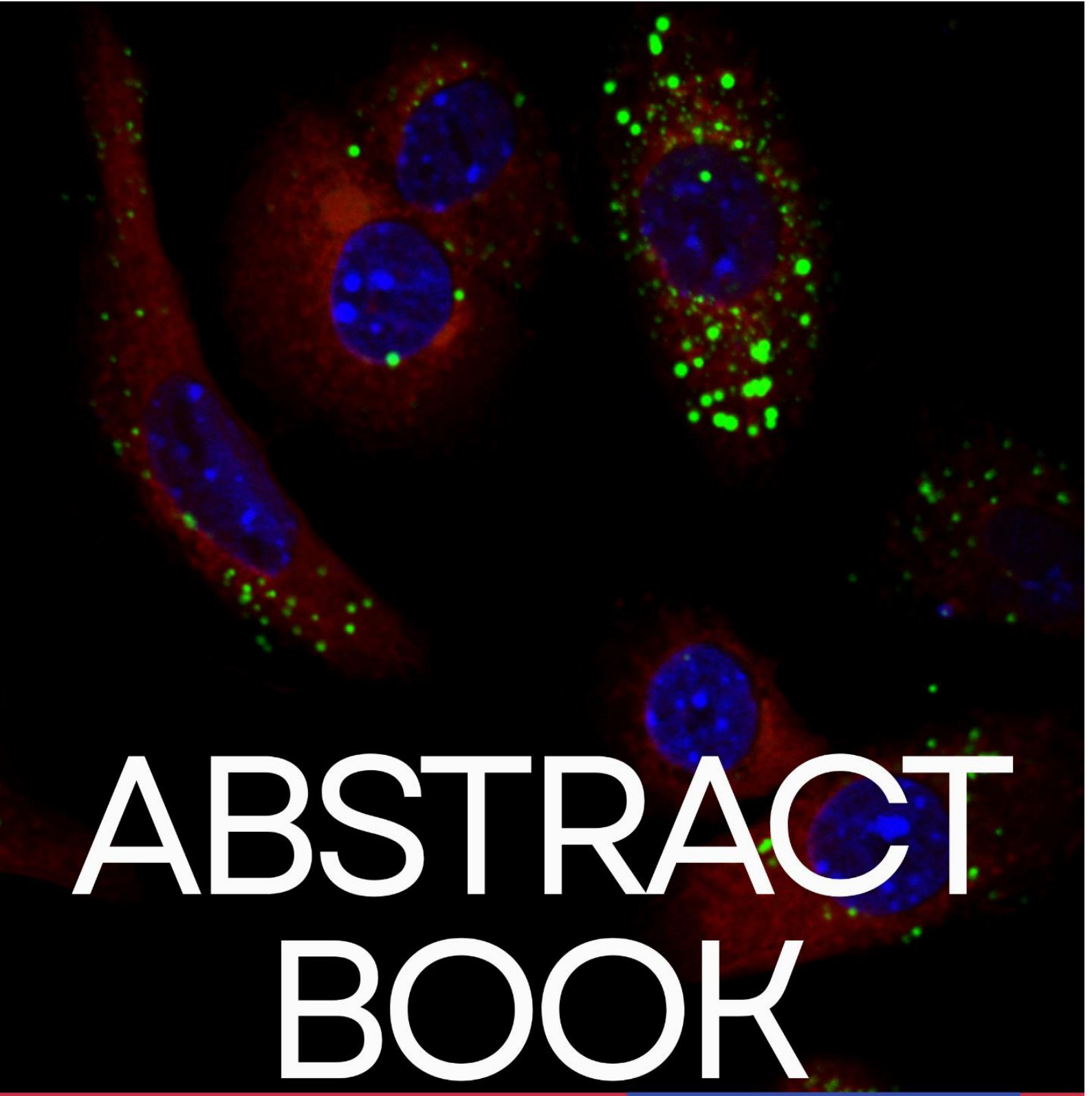


ABSTRACT BOOK

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Invited Lectures



A versatile "all-in-one" CRISPR-Cas13d tool for site-specific m6A demethylation in human mRNAs

Konstantina Athanasopoulou, Andreas Scorilas and Panagiotis G. Adamopoulos*

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N6-methyladenosine (m6A) is the most prevalent and dynamically regulated internal modification in eukaryotic mRNAs, critically influencing RNA stability, splicing and translation. Despite its biological significance, tools enabling precise manipulation of m6A at defined transcriptomic sites remain limited. Here, we introduce Dem6A-Vec, a single-plasmid, non-viral vector engineered for site-specific m6A demethylation in human mRNAs. Dem6A-Vec integrates all necessary components into one construct, eliminating the need for multiple plasmids or co-transfections. It expresses a catalytically inactive RfxCas13d (dCas13d) fused to the m6A demethylase ALKBH5, along with a programmable guide RNA (gRNA) driven by a U6 promoter. The construct carries HA and FLAG epitope tags for immunofluorescence-based localization and a nuclear export signal (NES) ensuring efficient cytoplasmic targeting of the fusion protein toward mature mRNAs. Using nanopore direct RNA sequencing, we identified 3,776 high-confidence m6A sites in HeLa cells, which served as potential Dem6A-Vec targets. Among these, EEF2 m6A:2886 and RRAGA m6A:418 exhibited high methylation stoichiometry (>0.8) and served as model targets for demethylation. SELECT-qPCR assays confirmed that Dem6A-Vec induced efficient and specific demethylation (>90%) at these loci without affecting adjacent m6A sites or other transcripts. Functional assays demonstrated that targeted demethylation increased mRNA stability, highlighting the regulatory impact of m6A removal. Importantly, Dem6A-Vec efficiently demethylated sites across a wide range of methylation stoichiometries, underscoring its adaptability to diverse transcriptomic contexts. Furthermore, the system supports reversible RNA modification, as methylation levels gradually recovered post-transfection, highlighting the reversibility and plasticity of RNA modifications. By integrating all necessary components into a single plasmid and enabling gRNA replacement, Dem6A-Vec represents a robust, scalable, and versatile platform for precise transcriptome engineering. This approach opens new avenues for dissecting the functional roles of m6A and other RNA modifications in gene regulation, cellular homeostasis, and disease, providing a foundation for future epitranscriptomic research and RNA-based therapeutic development.

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Photons for treating and detecting cancer

Prof. Mark Bradley
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Light comes in many different flavours and forms – it can be high energy as seen in x-rays or low energy in the form of near-infrared light – but all can be used within a chemical biology/medicinal context to interact with, and report-on disease. In my lecture I will talk of two-three areas of activity – all of which use photons to modulate or report on biological activity. This will include the ability to use light to modulate cellular/tissue function by synthesising within cells non-natural polymers, the ability to switch-on a variety of anticancer prodrugs (with deep penetration ability) or to light up disease.



Pharmacological Activation of NRF2: Redefining Therapy for MAFLD/MASH

Prof. Antonio Cuadrado

Department of Biochemistry, Medical College, Autonomous University of Madrid (UAM)

Background Metabolic dysfunction–associated steatohepatitis (MASH) is a complex and life-threatening liver disease with limited therapeutic options. Human transcriptomics indicates strong associations between NRF2-dependent genes and lipid metabolic pathways. The potential of mild NRF2 activation as a therapeutic approach remains underexplored.

Methods Transcriptomic datasets from human MASH biopsies were analyzed to assess NRF2-associated metabolic pathways. Parallel studies were performed in a Western diet + CCl_4 murine model. PHAR, a small-molecule NRF2 modulator predicted to disrupt β-TrCP/NRF2 interaction, was evaluated *in vivo* and in TGF-β-stimulated LX-2 stellate cells. Endpoints included NAS score, oxidative-stress markers, inflammatory genes, fibrosis signatures, and lipidomic profiling.

Results Human and murine datasets displayed convergent alterations in NRF2-regulated pathways, including β-oxidation, carnitine shuttle, oxidative phosphorylation, ROS detoxification, and acylglyceride metabolism. PHAR treatment prevented disease progression in the WD+CCl_4 model, reducing NAS scores and normalizing oxidative-stress, inflammatory, and fibrotic markers. Lipidomic analysis showed partial recovery of TAG, DAG, phospholipid, and sphingolipid profiles. In LX-2 cells, PHAR significantly attenuated TGF-β–induced fibrogenic activation, outperforming existing NRF2-targeting compounds.

Conclusion These findings support mild pharmacological NRF2 activation as a promising therapeutic avenue for MASH. PHAR demonstrates protective effects across oxidative stress, inflammation, fibrosis, and lipid metabolism, warranting further translational development.

Keywords

NRF2, MASH, lipid metabolism, steatosis, fibrosis, oxidative stress



Evolutionary diversification of quaternary structure

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Many enzymes form homomeric protein complexes. Sometimes there is a clear reason for this, when the active site is formed between subunits or when assembly is used as a means of allosteric regulation. But in many cases, enzymes have one active site per promoter and no known regulatory mechanisms that depend on self-assembly. This has lead theorists to propose that self-assembly may evolve neutrally. In this talk I will summarise our wok on the evolution of self-assembly in the citrate synthase family, which shows a remarkable plasticity in self assembly forms, including the first regular molecular fractal in nature.



Structure guided discovery of new generation antimalarial drugs

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Plasmodium falciparum, responsible for the most severe form of malaria, relies on an extensive repertoire of exported proteins to remodel mature erythrocytes and enable its intraerythrocytic development. Central to this process are exported heat shock proteins (Hsp70/Hsp40), which act as molecular chaperones supporting parasite protein folding, stabilization, and trafficking. Targeting these chaperone systems has gained considerable interest, as dynamic changes in the parasite's proteome represent a major barrier to durable antimalarial therapies.

In this study, we present an integrated structural biology and in silico strategy aimed at the rational design of next-generation antimalarial compounds. We focus on a detailed characterization of the PfHsp70-x catalytic pocket to identify and exploit distinct subsites that may accommodate highly selective small-molecule inhibitors.

Our findings pave the way for the development of selective inhibitors that specifically target the parasite's chaperone network while minimizing interference with human orthologues. Such compounds hold promise not only as potential therapeutic leads but also as molecular tools for dissecting the complex chaperone machinery within infected erythrocytes. Ultimately, this work supports the advancement of innovative intervention strategies aimed at sustaining and expanding global malaria elimination efforts.

Acknowledgements

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Persulfidation–phosphorylation crosstalk orchestrates white adipose tissue browning

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Persulfidation, a hydrogen sulfide (H_2S)-mediated post-translational modification, serves as a redox-protective mechanism that prevents irreversible cysteine overoxidation. Global ablation of the transsulfuration enzyme cystathionine-γ-lyase (*Cth*), a primary source of endogenous H_2S, resulted in upregulation of uncoupling protein 1 (*Ucp1*) and the formation of beige adipocyte clusters within inguinal white adipose tissue (iWAT), thereby promoting its browning. This remodeling was accompanied by enhanced mitochondrial biogenesis and elevated expression of glycolytic beige-selective markers, and was associated with reduced fat mass and improved glucose tolerance. The regulatory role of CTH on *Ucp1* expression was further validated pharmacologically using propargylglycine, a CTH inhibitor. Adipocyte-specific *Cth* knockout mice exhibited enchnced thermogenic fat formation and reduced body weight, confirming the cell-autonomous effects of CTH. *In-vitro* approaches using primary adipocytes demonstrated that browning occurs through transdifferentiation of white adipocytes.

The molecular events linking CTH deficiency to *Ucp1* upregulation were mapped through integrated multi-omics analyses, including transcriptomics, proteomics, persulfidomics, and phosphoproteomics. Loss of *Cth* markedly diminished global persulfidation of iWAT and resulted in a robust increase in protein phosphorylation. Mechanistically, inhibition of persulfidation of critical cysteine residues within the catalytic subunit of protein phosphatase 2A (PP2Ac) diminished its phosphatase activity. Downregulation of persulfidation was accompanied by enhanced phosphorylation and activation of GA-binding protein alpha chain (GABPA). GABPA direct binding to the proximal regulatory region of Ucp1 gene locus was confirmed by chromatin immunoprecipitation followed by sequencing. Thus, the PP2A-GABPA signaling axis underlies the metabolic and phenotypic reprogramming observed with CTH loss.

Our findings unravel the existence of a redox-sensitive cross-talk between two major posttranslational modifications, persulfidation and phosphorylation, that governs adipocyte plasticity. This mechanistic interplay drives white adipose tissue browning and identifies new molecular targets for therapeutic intervention to imporve metabolic health.



Decoding Amyloid Mechanisms: From Structural Principles to Therapeutic Engineering

Nikolaos Louros

Thomas O. Hicks Scholar in Medical Research, Assistant Professor of Biophysics Center for Alzheimer's and Neurodegenerative Diseases, Department of Biophysics, UT Southwestern Medical Center, Dallas, USA

Protein misfolding and amyloid formation are central to numerous degenerative diseases, yet the molecular determinants that govern their structural diversity, propagation, and pathogenicity remain elusive. We employ an integrated framework combining sequence-based mapping of aggregation-prone regions with thermodynamic and structural analyses of amyloid polymorphs to uncover unifying principles of assembly. By coupling these insights with advanced biophysical and cellular assays, we can dissect propagation dynamics and identify heterotypic interactions that modulate amyloid behavior across systems. Leveraging this mechanistic understanding, we are engineering de novo, conformation-selective inhibitors that target distinct amyloid architectures. This strategy establishes a rational, structure-informed path from the fundamental energetics of amyloid formation to the design of next-generation therapeutics aimed at controlling pathological aggregation.



Role of Senescence and Aging in Lung Carcinogenesis: Therapeutic tools

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Recent evidence highlights the importance of the tumour microenvironment (TME) to generate a permissive niche for cancer initiation and post-treatment relapse. Cellular senescence has been recognised as a cancer hallmark in response to persistent oncogene activation or genotoxic stress by playing numerous cell autonomous and paracrine tumour-promoting activities. Supported by successful preclinical validations in murine cancer models, the development of senotherapies is emerging with promise. However, final translation to human settings is still a significant challenge.

We have identified a population of tumour-associated macrophages (TAMs) in lung premalignant lesions that stimulate lung cancer onset and progression by secreting senescence-associated secretory phenotype (SASP) immonusuppressive factors **[1].** Genetic or senolytic ablation of senescent populations, or macrophage depletion following CSF1R blockade, result in a significant reduction in tumour burden and increased mouse survival of KRAS-driven lung cancer models. Of translational relevance, we reveal the presence of protumorigenic senescent macrophages in human lung premalignant lesions, including atypical adenomatous hyperplasia and adenocarcinoma in situ, but not in adenocarcinomas. Interestingly, we have found that aging drives a senescent tumour-promoting lung microenvironment (*Unpublished*). The combination of lung damage and aging creates a permissive niche for lung carcinogenesis.

In the case of advanced lung adenocarcinoma, we have found that a platinum-derived SASP enhances the malignant phenotype of nearby lung cancer cells **[2].** By using xenograft, orthotopic and KrasG12V-driven murine lung carcinoma models, we demonstrate that platinum-induced senescent cells strongly promote tumour progression. Mechanistically, we find that a TGF-β-enriched SASP drives pro-proliferative effects through TGFβR1 and Akt/mTOR signalling pathway activation. We validate the translational relevance of chemotherapy-induced SASP using clinical lung cancer samples from patients subjected to neoadjuvant platinum-based chemotherapy. Remarkably, by using distinct murine lung cancer models, we show that concomitant treatment of TGFBR1 inhibitors or senolytics with platinum-based chemotherapy reduces tumour burden and improves survival, thereby providing pre-clinical proof-of-concept for future trial designs.

Research in our laboratory has delivered a toolkit to target the lung tumour microenvironment consisting in (i) nanotechnologies and senolytic prodrugs (Nav-Gal) to eradicate senescent cells **[3,4]**, (ii) senoprobes (NanoJAGGs) to longitudinally monitor senescent cells by photoacoustic tomography (PAT) and fluorescence **[5]**, and transient reprogramming (by Yamanaka OSKM factors expression) inducing senescence and subsequent apoptosis of lung cancer cells **[6].**

Altogether, our results provide insights into the role and impact of senescent ecosystems in lung cancer, and propose potential therapeutic and detection modalities to target senescence for the management of lung cancer in the clinic.

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Mitophagy sustains inter-organellar communication to promote healthspan and lifespan

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Ageing and neurodegeneration are characterized by a progressive decline in the communication among key organelles, including mitochondria, the endoplasmic reticulum (ER) and lysosomes. Emerging evidence highlights mitophagy, the selective removal of damaged mitochondria, as a central modulator that sustains this inter-organellar crosstalk. Disruption of mitophagy leads to ER fragmentation, lysosomal dysfunction, impaired mitochondrial dynamics and disturbances in cellular homeostasis, underscoring the pivotal role of efficient mitochondrial turnover for the maintenance of global organellar architecture. Conversely, boosting mitophagy restores structural integrity and functional connectivity across subcellular compartments. Natural bioactive compounds, such as Urolithin A and spermidine, have recently drawn attention for their ability to enhance mitophagy and rejuvenate organellar interactions across taxa. Mitophagy activation mediates coordinated changes in energy metabolism, stress adaptation, and cellular resilience, ultimately supporting tissue functionality during ageing.

Our work demonstrates that mitophagy is crucial for preserving a functional organellar communication network, thereby safeguarding cellular homeostasis and viability. Enhancing mitophagy improves both healthspan and lifespan, establishing it as a promising target to tackle age-associated tissue deterioration and neurodegeneration. These findings emphasize the physiological importance of organellar crosstalk and point to mitophagy as a key determinant of organismal health.



Mechanisms of Lung Cancer Progression

Thales Papagiannakopoulos[1,2]

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KRAS mutant lung adenocarcinoma is the major form of lung cancer that remains a major challenge for clinical oncology because patients are refractory to standard-of-care. *KRAS* mutant tumors display a high degree of genetic heterogeneity and emerging clinical data suggest that specific *KRAS* co-mutations are associated with poor prognosis and failure of cancer therapies. Our group uses genetically engineered mouse models and patient samples to dissect how genetic subsets of *KRAS* mutant lung cancer promote tumorigenesis by rewiring cancer cell metabolism, promoting immune evasion and therefore contributing to tumor progression and therapy resistance. We have identified novel therapeutic approaches to both suppress tumor metabolism and reverse the immunosuppressive microenvironment of genetic subsets of KRAS-driven lung cancer.

Furthermore, our group is investigating how cancer as a systemic disease can lead to dysfunction of multiple organ systems, which is observed in many lung cancer patients. We are dissecting the interplay of tumor mutations, nutrition and changes in systemic physiology during tumorigenesis. Our studies highlight that genetic subtypes of *KRAS* mutant lung cancer can interact with common diets to disrupt systemic physiology. We aim to identify and therapeutically target the underlying mechanisms in order to restore homeostatic control of physiological functions.



Determinants of CRISPR/Cas9 Scission Profile for Precise, Predictable and Personalized Genome Editing

Vasileios Roukos

Assistant Professor of Biology, Department of Medicine, University of Patras

CRISPR/Cas9 is a powerful genome editing platform, with immense potential for facilitating the gene therapy of various diseases. Cas9 has a flexible scission profile, which might impact repair outcomes, however, what dictates the type of Cas9 incisions is largely unknown. We have recently developed BreakTag, a versatile, highly parallel and scission-aware methodology for profiling of Cas9-induced DNA double strand breaks (DSBs) and for identifying the determinants of Cas9 incisions. By assessing the end structures of more than 100,000 endogenous loci uniquely cleaved by SpCas9 across the human genome, we found that, in addition to frequent blunt DSBs, ~34% of SpCas9 cut sites form staggered DSB ends, displaying one to three nucleotide 5' overhangs. Training a machine learning model to predict Cas9 scission profiles revealed that Cas9 incision is highly dependent on the protospacer sequence, with guanine at position 17 or 18 favoring blunt or staggered incisions, respectively. Comparing matched datasets of Cas9 incisions and repair outcomes, we established that Cas9-mediated staggered breaks are linked with precise, templated and predictable single-nucleotide insertions, indicating that controlling these cut profiles might allow prediction of repair genotypes with desirable indels. We further demonstrate that common human genetic variation impacts the configuration of Cas9 cuts along with the repair outcome, and we identify Cas9 variants with altered scission properties. Our work illuminates the fundamental characteristics of the Cas9 nuclease and lays the foundation for harnessing the flexible cut profiles of Cas9 and engineered variants for precise and personalized, template-free, genome editing.



Antigen Presentation in the Tumor Ecosystem

Dr. Maria Tsoumakidou
BSRC Alexander Fleming
Medical School of the University of Athens

Diverse subsets of antigen-presenting cells (APCs) reside within solid tumors and have the potential to capture and present cancer antigens locally. The cellular and molecular interactions, as well as the challenges and outcomes of intratumoral cancer antigen presentation are poorly understood. Our laboratory has recently pioneered studies demonstrating that fibroblasts present cancer antigens to CD4 T cells within lung tumors. We proposed a model wherein intratumoral APCs sustain CD4 T cells independently of lymph nodes, known as the "Second Touch Hypothesis." Currently, we are exploring the landscape of lung cancer APCs and seeking to understand how the tumor microenvironment affects them. During the first part of my talk, I will showcase space-aware transcriptomic maps of mesenchymal APCs in human lung tumors. I will pinpoint two subsets of antigen-presenting mesenchymal cells with different organization patterns in tumor ecosystems: an alveolar epithelial–derived and a myeloid cell–derived. In the second part, I will reveal a double-edged sword function for type I interferons. Specifically, I will explain how interferon-inducible GBP-2 induces pyroptotic death in intratumoral dendritic cells. By elucidating the complexities of cancer antigen-presenting cells, my laboratory aims to pave new pathways to more effective cancer immunotherapies.



AI for Molecular Diagnostics and Biomedical Research: Transforming Medicine as We Know It !

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Professor and Vice Chair, Department of Laboratory Medicine and Pathobiology, University of Toronto, Canada

The use of artificial intelligence in healthcare is rapidly becoming a reality, and machine-learning models have already been approved for clinical diagnostics. As technologies evolve and their use becomes more widespread, the practical considerations for the clinical implementing artificial intelligence must be pragmatically assessed.

There are basic requirements for a successful AI model, including the availability of a large cohort of data for AI analysis. Legal consideration, including the need for informed consent and research ethics board approval are essential. Most AI algorithms require collaboration between institutions, and as such, safe sharing of data needs to be ensured.

In developing new AI models, there are multiple considerations, including paying attention to and reducing AI bias, and being aware of AI model degradation. There are multiple levels of regulation for the clinical usage of AI models, including FDA approval (or equivalent), lab-developed tests (LDT), and research use only. Multiple factors should be carefully assessed before the clinical use of AI, including cost-effectiveness, the need for rigorous validation, and careful assessment of the accuracy of AI models.

There are multiple caveats to using AI algorithms, including the over-reliance on AI algorithms with their negative impact on clinical care. Mitigating the risk of over-reliance on AI is a multidisciplinary effort. Finally, the reimbursement for the cost of AI algorithms and sources of funding has to be identified.


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ST1 - 121

Lon Protease Controls PPR-mediated RNA Processing in *Arabidopsis* Mitochondria

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Selective proteolysis is essential for cellular homeostasis, preventing the accumulation of unfolded or misfolded proteins that could otherwise form harmful aggregates. Lon, a highly conserved AAA^+ (ATPases Associated with diverse cellular Activities) protease, plays a central role in protein quality control across bacteria and eukaryotic organelles. However, the mechanisms underlying Lon-mediated substrate recognition and degradation in plant mitochondria remain poorly understood. Here, we identified mitochondrial substrates of the Arabidopsis Lon1 protease using a proteolytically inactive Lon1-trap variant expressed in a *lon1* mutant background, which exhibits growth retardation due to mitochondrial dysfunction. The captured substrates are involved in diverse mitochondrial processes, including energy metabolism, transcription and translation, highlighting the pivotal role of Lon1 in maintaining mitochondrial integrity. Notably, we identified a set of *bona fide* Lon1 targets, including enzymes involved in calcium transport, lipoic acid synthesis, heat shock responses, and Pentatricopeptide Repeat (PPR) proteins, which are known key regulators of mitochondrial gene expression. The PPR proteins were markedly overrepresented in the Lon1-trapped proteome, correlating with impaired group II intron splicing and RNA editing, particularly in transcripts encoding Complex I subunits, cytochrome *c* biogenesis factors, Complex IV components and ribosomal proteins. Collectively, our findings uncover a novel regulatory function for Lon1 in mitochondrial RNA processing and maturation, accomplished through the selective proteolysis of PPR proteins.

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ST2 - 138

Investigating the role of FspA/B proteins in lipid peroxidation, ferroptosis and antifungal sensitivity in *Aspergillus nidulans*

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Given the lipid-peroxidation-protective and ferroptosis-suppressive role of the ubiquinone reductase FSP1 in humans [1],[2], the physiological role of two *A. nidulans* orthologues (FspA/B) was investigated. Subcellularly, FspA showed abundant cytoplasmic and perinuclear localization, while FspB a less abundant localization in the cytoplasm.

Sensitivity to lipid peroxidation was analyzed by supplementation with fatty acids e.g. a-linolenic acid (LNA) and with copper (Cu), which is known to cause peroxidation in membrane lipids [3],[4]. No significant phenotypical differences among wild-type and overexpressing/knockout FspA/B strains in LNA-supplemented media were observed. Similarly, increased but similar reduction/oxidation levels of the lipid peroxidation sensor BODIPY™ were measured in wild-type and Δ*fsp*A/B single/double mutant strains in LNA-supplemented media, while no increase in oxidized lipids was shown in the presence of copper. Using a fluorometric assay, it was revealed that LNA produces similar ROS levels in wild-type and Δ*fsp*A/B single/double mutant strains**.**

Ferroptosis-suppressive role of FspA/B orthologues was investigated using growth tests in iron supplemented media. Our results showed normal growth of overexpressing/knockout FspA/B strains at low (1 mM) and severe growth defects at high (over 6 mM) iron concentrations of all strains tested. Addition of iron was shown to rescue/protect quiescent conidia from Cu deleterious impact on their growth in survival tests. Moreover, erastin, a glutathione-synthesis inhibitor/ferroptosis inducer, was shown not to induce ferroptosis of *A. nidulans* cells in the presence of LNA, Cu or Fe.

However, a significant correlation of FspB with resistance to the known antifungal amphotericin B (AmB) of *A. nidulans* cells was revealed, using drop tests as well as confocal microscopy experiments focused on the internalization of the lipophilic membrane dye FM4-64. Given the aforementioned, further experimental approaches to decipher the role of FspA/B in *A. nidulans* are in progress.

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ST3 - 218

The *Storekeeper* (*STK*) gene of tomato is a susceptibility factor in the Tomato yellow leaf curl virus (TYLCV) interaction

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Tomato yellow leaf curl virus (TYLCV) severely affects tomato (*Solanum lycopersicum*), one of the world's most important vegetable crops. To enhance viral resistance, the *Storekeeper* (*STK*) gene was selected as a potential host susceptibility gene, as it was found to be targeted by endogenous virus-activated small RNAs (vasiRNAs) and downregulated following TYLCV infection (unpublished data). *STK* silencing was first validated with Virus-Induced Gene Silencing (VIGS) using TRV vector. RT-qPCR analysis showed reduced *STK* transcript levels in plants agroinfiltrated with the pTRV1+TRV2-STK construct compared to the control, correlating with decreased viral accumulation and infection rates. These results suggest *STK* as a promising susceptibility gene for TYLCV resistance, and a suitable target for gene editing. CRISPR/Cas9 editing was then employed to improve tomato resistance to TYLCV. Single guide RNAs (sgRNAs) targeting *STK* were designed for CRISPR/Cas9-mediated mutagenesis. A ribonucleoprotein (RNP) assay confirmed specific double-stranded DNA cleavage *in vitro*. *In vivo* validation was performed via agroinfiltration of tomato cotyledons (cv. Ace), using a pTRANS210-based vector (Čermák et al., 2017) carrying Cas9 and the two designed sgRNAs. High-fidelity PCR and linear amplicon Nanopore sequencing confirmed the intended edits at *STK* target sites of tomato genome. Geminivirus-derived replicons (GVRs) were shown to enhance transient gene expression, and *in vivo* validation was repeated in the tomato cv. Micro-Tom using three GVRs and a non-replicating T-DNA. PCR screening revealed editing events (a 200 bp deletion in *STK*), with plants agroinfiltrated with Tomato leaf curl virus (ToLCV) GVR showing editing across all three replicates of the experiment at 7 dpi, aligning with earlier GVR experiments, where ToLCV GVR maintained high GFP expression in tomato, until 7 dpi. Nanopore sequencing confirmation is ongoing. These results demonstrate the utility of GVRs for CRISPR/Cas9 genome editing in tomato, highlighting their potential to enhance editing efficiency for functional genomics and tomato improvement.

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ST4 - 53

Comparative Analysis of Molecular Mechanisms Regulating Reprogramming in Distinct Stem Cell Types

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Aim: Induced pluripotent stem cells (iPSCs) can be generated from lineage-restricted somatic cells through overexpression of key transcription factors such as OCT4, SOX2, KLF4, and c-MYC, providing a versatile platform to study genetic, epigenetic, and environmental interactions. This study aimed to determine whether iPSCs derived from distinct lineages retain residual lineage-specific molecular signatures post-reprogramming and to delineate their established pluripotency networks.

Materials & Methods: iPSCs were generated from erythroblasts derived from CD34+ hematopoietic progenitors and from amniotic fluid-derived mesenchymal stem cells (AF-MSCs) using a polycistronic lentiviral system. Initial culture was performed on irradiated mouse embryonic fibroblasts (MEFs), followed by transfer to Matrigel-coated conditions. Subsequent proteomic and transcriptomic profiling was performed, using established protocols.

Results: Comparative analyses revealed >50% similarity in gene and protein expression profiles between the two iPSC lines, confirming efficient reprogramming while indicating partial retention of lineage-specific molecular features. Integrated multi-omics analyses including differential expression, transcription factor enrichment (Chea3), and database cross-validation identified 330 pluripotency-, 154 hematopoietic-, and 165 mesenchymal-related genes. Pathway enrichment analysis ($p<0,01$) further highlighted 74 genes involved in chromatin remodeling, epigenetic regulation, and differentiation (e.g., DNMT3A, CTCF, LIN28A, POU5F1) as potential core regulators of pluripotency. In mesenchymal-derived iPSCs, 31 genes were linked to canonical tri-lineage differentiation (osteogenic, chondrogenic, adipogenic) and fibroblast/hepatocyte-like pathways (e.g., FADS2, COL2A1, LIPA, NOTCH3). In erythroblast-derived iPSCs, 48 genes were associated with hematopoietic and erythroid lineage functions (e.g., JUND, JUNB, ITGA2B, TXN).

Conclusion: Our findings indicate that iPSCs retain a degree lineage-dependent molecular signatures despite the activation of core pluripotency networks. The integration of transcriptomic and proteomic datasets with comparative analyses across lineage-specific datasets enhances the biological relevance of our results and provides insights into potential molecular and epigenetic mechanisms underlying reprogramming fidelity and lineage memory. Ongoing studies will include chromatin accessibility profiling to further elucidate the epigenetic basis of these observations.



ST5 - 3

Evaluation of thermodurability and activity changes of PET-hydrolase variants in a directed evolution pathway

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Enzymatic recycling of PET plastic requires enzymes with high stability at high temperatures. *Ps*PETase degrades PET polymers at ambient temperatures. HotPETase, designed with directed evolution, is a thermotolerant variant of *Ps*PETase, able to degrade PET at 65°C.
In this project, experimental and computational approaches were used to investigate the stability of these two enzymes, as well as their intermediates in the directed evolution trajectory. Protein unfolding was studied with differential scanning calorimetry. The PET degradation rates of all enzymes were determined with small-scale activity assays. Large-scale degradation reactions were performed using PET powder or film as substrates. Binding of PET to the active site was investigated with molecular docking calculations. The best docked poses were further refined with molecular dynamics simulations to account for flexibility and temperature effects.
Activation energy ($^{(E_{act})}$) and activation temperature ($^{(T_{act})}$) showed an increase during the first rounds of the evolution process. HotPETase and one predecessor (M10) displayed increased thermodynamic properties. The optimum temperature for activity increased from *Ps*PETase to HotPETase, with activity assays revealing different kinetic stabilities under different conditions. HotPETase displayed a dependency to the BugBuster detergent, which was present in the evolution screening process, and affected differently catalysis on small- and large-scale. Analysis of molecular dynamics simulations of the variants indicated the probability of PET obtaining distances, necessary for catalysis, within the active site. For simulations at 60°C, HotPETase and M10 were found to have probability of obtaining those distances, whereas *Ps*PETase was incapable of.
This study was the first to our knowledge to provide a thorough investigation of a directed evolution pathway of PET-hydrolases. It was demonstrated that a small number of mutations can have a significant impact to thermodurability, but unexpected dependencies can also occur due to the random nature of the evolution approach. The molecular dynamics simulations were able to capture the thermodurability increase of the evolved variants at the high temperature simulations.

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ST6 - 32

Discovery of novel antimicrobials: Inhibitors of functional amyloid aggregation linked to biofilm formation

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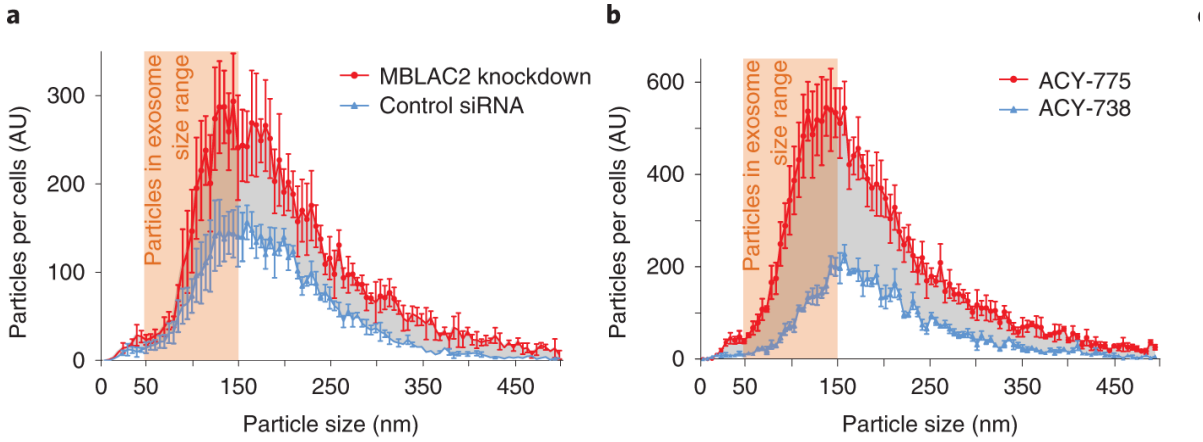
Bacterial biofilms are major contributors to chronic and recurrent infections due to their high tolerance to antibiotics. In *Escherichia coli*, biofilm stability relies heavily on curli fibers, functional amyloids assembled from the major subunit protein CsgA. Targeting these amyloids represents a promising strategy for next-generation antimicrobials (NGAs), which act at sub-lethal concentrations to inhibit virulence, enhance antibiotic susceptibility, and reduce the risk of resistance development. Here, we applied a bacterial fluorescence-based screening platform, combined with fluorescence-activated cell sorting and deep sequencing, to identify cyclic peptides that inhibit CsgA aggregation. Several candidates emerged as potent inhibitors of amyloid and biofilm formation. Their activity was validated by crystal violet biofilm assays, Congo red staining, and microscopy, which consistently demonstrated reduced amyloid and biofilm formation in *E. coli* BW25113. Additionally, the cyclic peptides inhibit CsgA-dependent pellicle formation for the uropathogenic *E. coli* strain UTI89. *In vitro* Thioflavin T assays confirmed direct inhibition of CsgA aggregation. Other ongoing biophysical and biochemical assays are characterizing the mechanisms of peptide-mediated CsgA inhibition, and current studies are assessing their capacity to disrupt pre-formed biofilms and potentiate antibiotic action. These findings highlight functional amyloids as attractive therapeutic targets and demonstrate the effectiveness of our discovery pipeline in identifying novel aggregation inhibitors. Ultimately, cyclic peptide-based NGAs may provide new avenues for the treatment of persistent biofilm-associated infections.

ST7 - 219

MBLAC2 !?

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We serendipitously discovered MBLAC2 to be an off-target of a large part of the HDAC pharmacopoeia. The unexpected cross-reactivity with this small (279 amino acids), single domain, poorly characterized metalloprotein led us to investigate its role and to develop chemical probes. Despite its name, given by homology with other proteins of the MBL-fold, metallo-β-lactamase domain-containing protein 2 (MBLAC2) acts as an acyl-CoA hydrolase *in vitro*. We found MBLAC2 KO mice to develop normally, with no substantial physiological differences observed across >70 tested phenotypes. The proteomics of their brains, compared to wild-type mice, did not show significant differences in protein abundances other than MBLAC2, suggesting that MBLAC2 function is dispensable in healthy mice. However, in human cell lines, we discovered that pharmacological inhibition or knock-down of MBLAC2 lead to accumulation of extracellular vesicles (EVs), concomitant with changes in the intracellular lipidome. Considering the diverse physiological roles of EVs in cancerous and neurological diseases, the mapping of a new protein to vesicle biology opens exciting intervention avenues.



Related publications:

- **Target Deconvolution of HDAC Pharmacopoeia Reveals MBLAC2 as Common Off-Target**. *Nat. Chem. Biol.* **2022**, 18:812.

- Serendipitous and Systematic Chemoproteomic Discovery of MBLAC2, HINT1, and NME1-4 Inhibitors from Histone Deacetylase-Targeting Pharmacophores.
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ST8 - 100

Calcium- and Strontium- Doped Mesoporous Silica Nanoparticles: Linking surface design to Hemocompatibility and Coagulation Dynamics

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The present study aimed to elucidate how the physicochemical characteristics of mesoporous silica nanoparticles (MSNs) enriched with calcium (Ca) and strontium (Sr), influence their hemolytic and hemostatic behavior.

Materials and Methods: Sr- and Ca-enriched mesoporous silica nanoparticles (Sr–Ca–MSNs, MCM-41 type) were synthesized via the sol–gel method using CTAB, TEOS, and nitrate salts ($Ca(NO_3)_2{\cdot}4H_2O$, $Sr(NO_3)_2$) with 2.5,5.0 and 7.5 mol% Sr. Characterization was performed by SEM, TEM, FTIR, and NTA. Peripheral blood from ten donors was incubated (2 h, 37 °C) with Sr-Ca-MSNs (0.25,0.50 and 1.00 mg/mL). Hemolytic, oxidative, and mechanical effects were assessed. Flow cytometry evaluated PS exposure, iCa^{2+}, caspase-3, iROS, and P-selectin. Erythrocyte morphology, hemostatic parameters (PT, aPTT, fibrinogen, D-dimers, protein C, PPL, ATIII), and thromboelastometry were analyzed to determine clot properties and vesiculation. Data were processed with SPSS v27.

Results: Sr–Ca–MSNs (~100 nm) exhibited good dispersion and colloidal stability. Hemolytic activity increased dose-dependently, remaining within nanotoxicity limits (<5%) at lower concentrations. At 1.00 mg/mL, higher hemolysis occurred for Sr 2.5% (6.37±3.22%, $p<0.0001$) and Sr 5.0% (6.65±3.17%, $p<0.0001$) versus controls (0.53±0.36%). Oxidative hemolysis, iROS, and aging markers increased in a dose-dependent manner, while osmotic and mechanical resistance were unchanged. Coagulation tests demonstrated reduced PT/aPTT, decreased fibrinogen (Sr 5.0%, $p<0.01$), and elevated D-dimers ($p<0.0001$). Protein C rose significantly, whereas PPL and ATIII were unaffected. Thromboelastometry revealed shorter clot times (NATEM, INTEM), indicating faster clot initiation. Platelet activation increased at all Sr levels, and larger microvesicles (>300 nm) appeared at 0.50 mg/mL (Sr 5.0%, $p<0.01$).

Conclusions: Sr–Ca–MSNs exhibited favorable hemocompatibility at low doses and a dose-dependent procoagulant effect mainly via the intrinsic pathway. Their physicochemical structure appeared to enhance fibrin polymerization, platelet activation, and overall clot stability. These findings identify Sr-Ca-MSNs as promising hemostatic nanomaterials requiring dose optimization and in vivo validation.



ST9 - 11

Proteomic and Morphological Reprogramming of Breast Cancer-associated Adipocytes

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The tumor microenvironment (TME) is increasingly recognized as a dynamic regulator of breast cancer progression, with adipocytes functioning as active contributors rather than passive bystanders. The reciprocal interaction between breast cancer cells and cancer-associated adipocytes is indicative of sustained paracrine crosstalk, which modulates the behaviour of both compartments. In the present study, we investigated the proteomic and morphological reprogramming of breast cancer-associated adipocytes (BrCAAs) in response to triple-negative breast cancer (TNBC). An *in vitro* BrCAA model was established using conditioned medium from HCC1143 TNBC cells, followed by mass spectrometry-based proteomic analysis. Comparative profiling revealed 256 differentially expressed proteins, enriched for pathways including fatty acid degradation, carbon metabolism, and glycogen turnover, consistent with a metabolic shift from energy storage to energy supply. Gene ontology and protein-protein interaction analyses further identified cytoskeletal remodeling, adhesion dynamics, and secretory pathway activation, supporting BrCAA-driven microenvironmental remodeling. To investigate BrCAA morphologic changes *in vivo*, we used the MMTV-PyMT mouse model of breast carcinoma coupled to digital pathology. Morphometric analysis demonstrated progressive size reduction and increased contour irregularity of adipocytes adjacent to tumors, correlating with proteomic evidence of metabolic stress. Importantly, BrCAAs localized at tumor interfaces that were associated with increased microvessel density and CD105+ endothelial cells, consistent with their activation, compared to desmoplastic zones. Indeed, proteomic enrichment analysis highlighted pro-angiogenic remodeling, with validation of Basigin (BSG), Integrin αV (ITGAV), and 2,4-dienoyl-CoA reductase 1 (DECR1), as novel proangiogenic mediators. Collectively, our findings identify candidate mediators of BrCAA function and establish BrCAAs as metabolically and structurally reprogrammed stromal components that promote tumor metabolism and localized angiogenesis, representing potential therapeutic targets in aggressive breast cancer.



ST10 - 204

Origins and evolution of protein novelty across the entire budding yeast subphylum Saccharomycotina / an ancient yeast lineage

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Novel protein-coding genes are important drivers of evolutionary adaptation and often underlie species-specific characteristics. We now know that novel genes can emerge entirely de novo from previously non-coding genomic sequences, yet much about this process as well as its overall evolutionary importance remain unclear. Here, we conducted the largest-scale computational investigation of de novo gene evolution to date, exploiting a dataset, which is comprised from 332 Saccharomycotina genomes spanning the entire biodiversity of the yeast subphylum. We applied a multi-layered, stringent methodology to pinpoint the branches of origin of all Saccharomycotina subphylum gene families, focusing on the ones that are subphylum-specific. We established a robust set of 5,662 de novo evolved genes, most of which species-specific, with those found in multiple species bearing strong signature of purifying selection. Only a small percentage of these genes could be explained by Horizontal Gene Transfer from prokaryotes, suggesting that the majority of these genes (XX%) likely originated de novo. We also inferred that more than 20,000 novel genes originated through extreme sequence divergence and that they differ in their sequence and structural properties from those evolved de novo, revealing two distinct modes of evolution of novelty. Our analysis of how gene and protein properties change throughout evolution revealed that some, like GC content, remain remarkably similar, while others, like intrinsic disorder vary significantly. At the same time, we demonstrate that such evolutionary trends are heavily influenced by the level at which gene families are clustered. Lastly, a detailed analysis of predicted tertiary structures of de novo proteins revealed multiple cases of entirely novel folds and intriguing cases of potential de novo convergence, while highlighting the limitations in terms of prediction confidence, for evolutionarily young, short proteins. Our work paints a comprehensive picture of how protein novelty can originate and evolve through different routes, at an unprecedented taxonomic scale.



ST11 - 133

Deep proteomic exploration of bone marrow CD138+ cells from 200 MGUS, SMM and multiple myeloma patients

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Monoclonal gammopathy of undetermined significance (MGUS) is present in 3-5% of the aging European population, and 1% progress to multiple myeloma (MM) annually. As the disease eventually develops resistance to all available treatments, it is crucial to understand the mechanisms involved in the progression from MGUS or smoldering MM (SMM) to active MM to inform monitoring strategies and find new therapeutic targets. CD138+ cells isolated from bone marrow (BM) are composed of polyclonal ('normal') and clonal ('diseased') plasma cells, the latter in increasing proportion as disease progresses. Using a state-of-the-art mass spectrometry pipeline, we aimed at identifying protein signatures characteristic of clonal cells infiltrating the BM of patients with asymptomatic vs symptomatic disease.

We carefully selected 200 patients with MGUS (75), SMM (56) or MM (69) and biobanked CD138+ cells. We tailored a bottom-up proteomics pipeline using the protein phase after trizol extraction[1]. Nanoflow LC-MS/MS (data independent acquisition) was used to acquire high-depth proteomic profiles later pre-processed using FragPipe. After thorough quality assessment and analysis of confounders, we compared proteins identified in the groups. We analysed their association with the clonal cell proportion in each sample (inferred from routine flow cytometry analyses), and with other routine clinical metadata and blood tests results.

This unique sample set allowed us to identify over 7000 proteins and determine the main confounders to be addressed during study design and data analysis. Cell profiles of MGUS and SMM patients were similar, and we achieved better separation using SMM risk profiling as defined by the IMWG[2]. We will present the impacted proteins and pathways in relation to the hypotheses currently discussed in the literature. This study is the largest assessing BM CD138+ cell profiles at protein level, providing robust data to better understand the metabolic and immune system shifts observed from MGUS to MM stages.

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ST12 - 24

Splicing modulation of the lncRNA PVT1 and its effect on regulation of target gene expression

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Long non-coding RNAs (lncRNAs) are a large group of non-coding RNAs, involved in fundamental processes, such as genome organization, chromatin remodeling and gene expression regulation. However, the role of their splicing activity is not yet clearly understood. Machine learning models have identified that splicing efficiency is one of the parameters driving chromatin dissociation of lncRNAs, while among the transcripts that remain chromatin-associated, is the plasmacytoma variant translocation 1 (PVT1)[1]. In this study, we utilize the technology of CRISPR Artificial Splicing Factors (CASFx)[2] to artificially modulate splicing of PVT1 in luminal A breast cancer cells. PVT1 may have oncogenic role in breast cancer[3], and its dysregulation has been associated with several other types of cancer as well. PVT1 shows decreased splicing efficiency of 3' splice sites in breast cancer cell lines compared to normal breast cell lines, as well as containing perfect (7-mer) miR-200 seed sites exclusively within its intronic regions. This would enable it to function as a chromatin-associated RNA with intron-retained competing endogenous (ceRNA) activity. Here, we suggest a model in which artificial enhancement of PVT1 splicing efficiency leads to increased intronic turnover and affects the miR-200 regulation axis[4]. This study sets the base of developing options for RNA-based therapeutics that involve the targeted modification of abnormal splicing, considering that splicing dysregulation is a hallmark of cancer[5].

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ST13 - 9

TCRζ-Driven Pre-Signaling Organization of Lck in Rab11^{+} Endosomes Shapes TCR Activation

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T cell activation relies on the precise spatiotemporal regulation of TCR signaling at the Immunological Synapse, where vesicular trafficking coordinates the delivery of key signaling molecules. While endosomal pools of Lck and its immediate substrate, the TCRζ chain, have been associated with TCR signaling competence, the mechanisms underlying the regulation and synchronization of their trafficking routes remain unresolved. In this study, we simultaneously traced the endosomal localization dynamics of Lck, ζ chain and the TCR in unperturbed cells and under conditions that preserve the endosomal network integrity. We identified a previously unrecognized plasma membrane resident pool of ζ that exists independently of the TCR complex yet remains competent for phosphorylation and ZAP-70 recruitment. This "standalone" ζ population drives the recruitment of Lck and CD45 into Rab11-positive endosomes, establishing pre-assembled, signaling-primed platforms that could provide the means of sustained signaling without requiring continuous receptor engagement. Our findings advance the understanding of how vesicular ζ and Lck pools coordinate to shape TCR activation threshold and signaling persistence. Beyond the basic understanding of TCR signaling, these insights hold translational importance for optimizing the design and signaling efficiency of T cell immunotherapies.

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ST14 - 67

The extracellular matrix stiffness impacts lung cancer cell behavior and drug response

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Lung cancer remains the leading cause of cancer-related mortality worldwide. Advances in targeted therapies, such as EGFR inhibitors like Osimertinib, have transformed the treatment of certain subsets of lung cancer; nevertheless, the clinical benefit is still limited due to the emergence of resistance.
The tumor microenvironment in lung cancer can become increasingly stiff and fibrotic. While increased matrix stiffness is associated with cancer progression its role in shaping lung cancer cell behavior and therapy response remains incompletely understood.
Here, we used polyacrylamide hydrogels with tunable stiffness to investigate how lung cancer cells (PC9 and HCC4006 - EGFR-mutant; H292 – EGFR-wild type) respond to their mechanical environment. Cells sensed substrates of up to 0.5 kPa as soft adopting a smaller and rounder shape, whereas matrix as low as 2 kPa was perceived as stiff. Interestingly, the mechanosensitive YAP nuclear accumulation showed cell line-specific response. Transcriptomic analysis of cells grown on soft versus stiff substrates revealed differential expression of genes, including certain mucins as well as regulators of actomyosin contractility and microtubule dynamics. In addition, pathways associated with proliferation and epithelial–mesenchymal transition (EMT) were downregulated on soft matrices.
Upon drug treatment, a subset of cells survives by entering a reversible drug-tolerant state. We found that drug-tolerant cells emerge on both soft and stiff matrices, but rely on distinct adaptive mechanisms depending on the mechanical context. Combined inhibition of alternative signaling pathways revealed that MAPK pathway inhibition enhanced osimertinib efficacy across both conditions, whereas YAP/TEAD inhibition improved response only on stiff substrates, indicating that YAP-driven adaptation is stiffness dependent. To further dissect these adaptive programs, we performed transcriptomic profiling on soft versus stiff ECM, uncovering stiffness-specific resistance signatures.
Our findings suggest that ECM stiffness shapes lung cancer cell behavior and drug sensitivity, with implications for understanding resistance development in different tumor microenvironments.

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ST15 – 156

Cytoplasmic Galectin-1 is a novel luminal cargo of Weibel–Palade bodies, revealing a new facet of protein secretion

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Weibel–Palade bodies (WPBs) are the main secretory organelles of endothelial cells, responsible for the regulated release of von Willebrand factor (VWF), the key hemostatic protein, along with other vascular mediators secreted into the bloodstream upon stimulation[1]. In this study, we identify Galectin-1 (Gal-1), a cytoplasmic lectin typically secreted through unconventional routes[2], as a new luminal cargo of WPBs in HUVECs.

Mass spectrometry of the endothelial secretome first indicated the presence of Gal-1 in the conventional secretory pathway, which we confirmed by confocal microscopy, demonstrating that Gal-1 is stored in WPBs. Interestingly, Gal-1 was detected only in a subset of vesicles and the fraction of Gal-1–positive WPBs differed between HUVEC isolations, indicating regulated recruitment.

Since Gal-1 is cytoplasmic, it could localize either to the cytosolic surface of the WPB membrane or within the lumen. However, resolving this is technically challenging, as the diameter of WPBs (100–300 nm) lies well below the ~500 nm diffraction limit of confocal microscopy[3], preventing discrimination between luminal and membrane-associated signals using conventional imaging. To address this limitation, we applied three independent approaches: (i) vesicle reshaping, from elongated to spherical geometry to increase their diameter, (ii) super-resolution structured illumination microscopy (SIM^2, Zeiss Elyra 7) and (iii) Ultrastructure Expansion Microscopy (U-ExM). All three approaches converged on the same result, that **Gal-1 is localized within the lumen of WPBs**.

In addition, we investigated why Gal-1 is stored only in a subset of vesicles. Our results indicate that **Gal-1–positive WPBs may exhibit membrane damage**, consistent with the role of Gal-1 as a sensor of membrane damage in other types of vesicles, such as lysosomes[4].

Overall, our findings identify Galectin-1 as a new luminal cargo of WPBs and advance understanding of endothelial secretion, given its known ability to bind and modulate the activity of VWF[5].

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ST16 - 142

Nuclear rupture in confined cell migration triggers nuclear actin polymerization to limit chromatin leakage

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Upon cell migration in confined space, such as during cancer metastasis, mechanical forces from the extracellular matrix act onto the nucleus leading to nuclear envelope (NE) rupture, chromatin leakage and genomic instability. Here we found that during confined migration, NE rupture triggers dynamic nuclear F-actin formation dependent on the formins DIAPH1 and DIAPH3. We show that DIAPH3 dynamically and transiently relocates to the nucleus upon NE rupture. Interfering with DIAPH1/3 or with nuclear actin polymerization resulted in nuclear instability during confined migration. Notably, nuclear formin activity or actin assembly limit NE rupture-induced chromatin leakage. Similarly, silencing of Ataxia Telangiectasia and Rad3-related protein (ATR) reduced NE rupture-triggered nuclear F-actin assembly and increased chromatin leakage. Consistent with this, ATR promotes the phosphorylation of DIAPH3 at S1072 adjacent to its autoregulatory domain to promote nuclear actin polymerization. Using atomic force microscopy, we found that nuclear actin assembly or nuclear DIAPH3 activity promotes nuclear stiffness in an ATR-dependent manner. Thus, our study identifies an ATR-formin module that regulates nuclear mechanical properties through induction of intranuclear actin scaffolding.



ST17 - 8

Zincore, an atypical coregulator, binds zinc finger transcription factors to control gene expression

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Zinc finger proteins (ZNFs) are the largest family of transcription factors, yet how they activate gene expression and whether they require dedicated coactivators remains unclear. I will discuss the identification of Zincore, a previously uncharacterized protein complex consisting of QRICH1 and SEPHS1, as a novel ZNF-specific coactivator. Both QRICH1 and SEPHS1 are critical for embryonic development in mice and their haploinsufficiency is linked to similar neurodevelopmental defects in humans. We show that among Zincore clients, ZFP91 is a transcription factor that binds to the evolutionary conserved motif CTTTAAR, and the cryo-EM structure of Zincore in complex with ZFP91 bound to this motif. The structure reveals Zincore to recognise the DNA-bound zinc finger domains, explaining the recognition of a wide variety of ZNFs. I will show, also in the light of unpublished data on ZNF652, how this interaction stabilizes the binding of Zinc Fingers to the DNA, thereby locking them on consensus motifs, providing a unique activity for Zincore as a transcriptional coactivator. I will also discuss the use of protein language models to classify transcription factors and the potential of such methods to discover new functional annotations, especially in light of deciphering the Zincore interactome.

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ST18 - 287

The role of Transgelin in Gut Resilience and Epithelial Homeostasis in Drosophila

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The intestine of *Drosophila melanogaster* consists of a single-layered epithelium where enterocytes project densely packed, finger-like membrane protrusions called microvilli into the lumen, forming the brush border. These apical structures are mechanically supported by a highly organized actin cytoskeleton assembled directly beneath the plasma membrane, which not only provides structural integrity but also scaffolds essential molecular machinery for nutrient absorption, metabolic regulation, and immune surveillance. The intestinal epithelium maintains organismal homeostasis through integrated control of these processes, with the brush border maximizing absorptive surface area while coordinating metabolic homeostasis and innate immune responses. However, how the actin cytoskeleton mechanistically integrates with metabolic pathways and immune response regulation remains poorly understood.

Transgelins are evolutionarily conserved actin-binding proteins, and they have recently been identified as major regulators of lipid and anti-cancer function in $CD8^+$ T cells, yet their mechanistic role in epithelial physiology remains poorly understood (Nature, 2024).

In this study, we have focused on the Transgelin Chd64 protein, which is highly expressed in the gut epithelium (Vakaloglou et al., 2021). To elucidate the functional role of Chd64, we generated CRISPR-indel null mutants and employed an integrative multi-scale approach combining high-resolution confocal and transmission electron microscopy for ultrastructural analysis, alongside transcriptomic profiling via RNA-sequencing. Through these analyses, we identified that Chd64 is essential for maintaining gut epithelial architecture, metabolic homeostasis, and immune responses. Our ongoing work aims to delineate the molecular mechanisms by which Chd64 coordinates epithelial structural integrity, metabolic regulation, and antimicrobial defense, thereby establishing a tractable genetic model for studying intestinal brush border pathologies, metabolic dysregulation, and host-microbe interactions.

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ST19 - 70

Monocyte/macrophage profiles reflect cardiac unloading and heart failure state.

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Heart failure (HF) is the final effect of multiple converging pathways of inadequate repair following cardiac injury or stress. Upon left ventricular assist devise (LVAD) implantation end-stage HF patients may show signs of reverse remodeling and other beneficial responses. Despite recent advances the underlying mechanisms have not been yet clarified. We have previously shown that macrophages and their secretomes affect post-injury cardiac remodeling and associated outcomes. We hypothesized that peripheral immune status and particularly the monocyte/macrophage arm of the innate immunity system may both reflect and get affected by LVAD-mediated cardiac unloading. To test that we analyzed gene expression profiles of monocytes from LVAD receivers. We found that a series of inflammatory pathways were down-regulated during LVAD stay, including NFkB, TNFα, IL17, complement and NOD-like receptor signaling. Calcium signaling and cell death pathways were also affected. Intriguingly, GROα and IL-8, as well as their receptors CXCR1 and CXCR2 were down-modulated identifying potential inflammatory axes suppressed by the LVAD regime. Additional molecular players representing suppression of inflammation, as well as a limited number of up-regulated pro-inflammatory genes point out a complex pattern of regulation. STAT3, a master regulator of macrophage polarization, was also differentially expressed. Consequently, we further explored the polarization potential of macrophages derived from monocytes during in vitro differentiation. In line with the monocyte RNA-Seq data, RT-PCR studies revealed that during LVAD stay monocyte-derived macrophages from HF patients were more prone to follow alternative/pro-repair (M2) than pro-inflammatory (M1) polarization, suggesting an overall switch from pro- to anti-inflammatory potential. Importantly, the responsiveness to M1 or M2 stimuli of human macrophages derived from patients at the time of LVAD implantation correlated well with echocardiographic parameters, further suggesting that the systemic innate immune status not only reflects and responds to, but it may also contribute to the ongoing HF status and progress.

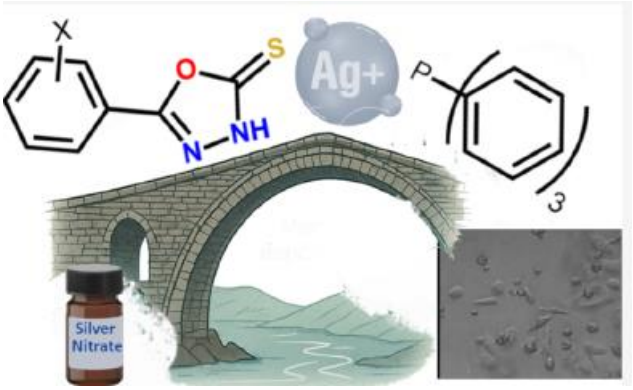
Supported by the Onassis Foundation (IΩ003 joint BRFAA-OCSC grant)

ST20 – 33

Mitochondria-Targeted Silver – Oxadiazole Conjugates as Potent Anticancer Agents

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Oxadiazole-2-thione derivatives have emerged as a fascinating class of bioactive molecules with the potential to modulate key cellular processes involved in disease. Their molecular framework supports a remarkable range of biological activities—including anticancer, antimicrobial, and anti-inflammatory effects—making them highly attractive leads for therapeutic development. The 1,3,4-oxadiazole core is already found in several clinically used drugs, such as zibotentan (targeting tumor progression), furamizole (antibacterial), and raltegravir (HIV antiviral), underscoring its biomedical importance. Yet, their molecular mechanisms of anticancer action remain largely unexplored, particularly when these ligands are integrated into metal complexes that can interact with DNA, enzymes, and mitochondrial targets. Unveiling these interactions opens new perspectives for rational design of metallodrugs that selectively [1].
In this study, a new family of silver(I)–oxadiazole–triphenylphosphine conjugates was synthesized as mitochondria-targeted anticancer agents. Structural analysis by X-ray diffraction revealed stable heteroleptic complexes with tunable geometry depending on the electronic nature of halogen substituents. These compounds exhibited potent and selective cytotoxicity against both hormone-dependent (MCF-7) and hormone-independent (MDA-MB-231) breast cancer cells, showing up to tenfold higher efficacy than cisplatin, while remaining nongenotoxic and nontoxic to normal cells. Mechanistic investigations demonstrated that they induce apoptosis through mitochondrial disruption, DNA interaction, and lipoxygenase inhibition, highlighting a strong structure–activity relationship that establishes silver(I)–oxadiazole conjugates as a promising class of bioinspired chemotherapeutic candidates [1].



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ST21 - 51

Analyses of proteostatic network alterations in Multiple Myeloma

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Multiple Myeloma (MM) is a chronic hematologic malignancy characterized by gradual progression, recurrent relapses, and eventual resistance to existing therapies. Despite major advances in understanding the molecular mechanisms underlying disease onset, recurrence, and therapeutic response, critical aspects of MM pathology remain insufficiently explored. To address these gaps, our study focuses on the proteostasis network (PN) [being composed, among others, from the endoplasmic reticulum unfolded protein response (UPR), molecular chaperones, the autophagy-lysosome (ALP) and the ubiquitin-proteasome (UPP) pathways], a critical regulator of proteome homeodynamics, often dysregulated in carcinogenesis, as well as on redox perturbations which influence the interplay between protein quality control and disease progression. By comparatively analyzing several MM cell lines representing distinct genetic backgrounds against a near-normal B lymphocyte line, we observed activation of UPP and ALP pathways in malignant cells, accompanied by accumulation of ubiquitinated and aggregated proteins indicative of enhanced proteome instability and proteotoxic stress. These molecular adaptations were associated with increased oxidative load and altered metabolic profiles, possibly reflecting the elevated bioenergetic and biosynthetic demands of MM cells. High-resolution proteomic analyses revealed differential expression signatures between MM and control cells, highlighting deregulated proteostatic, metabolic, secretory, and oxidative-stress-related pathways. Importantly, complementary analyses performed on patient-derived (MGUS), smoldering multiple myeloma (SMM), and overt MM revealed similar patterns of UPP and ALP activation as well as elevated oxidative stress markers underscoring the translational relevance of our *in vitro* findings. Our results highlight novel insights into how MM cells manipulate proteostatic pathways, offering a deeper understanding of disease mechanisms and paving the way for improved therapeutic strategies targeting PN modules in this hematologic malignancy.

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ST22 - 137

Polyunsaturated fatty acid-derived lipid mediator networks determine viral pneumonia severity and risk for critical COVID-19

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Severe respiratory infections such as COVID-19 are characterized by excessive inflammation leading to the development of pneumonia and acute respiratory distress syndrome. Bioactive lipid mediators (LMs) derived from ω6 and ω3 polyunsaturated fatty acids are central to the regulation of inflammation, controlling both its initiation and resolution. Still, their role in viral infections remains underexplored. By employing a holistic approach involving the analysis of white blood cell transcriptomes, targeted lipidomics, cytokine and immune cell profiling, we now show that LM networks are profoundly altered in COVID-19, correlate with inflammatory patterns, and stratify patients according to disease severity. Central to this are CYP450-derived LMs, such as 20-HETE, and lipoxygenase-derived LMs such as 15-HETE, both exhibiting vasoactive function, along with lipid peroxidation metabolites such as 10-HDOHE. Among them, 20-HETE appears to be a promising prognostic biomarker for ICU admission and a potential therapeutic target for severe COVID-19 disease. Our study emphasizes the importance of LM networks in COVID-19 pathophysiology and sheds light into the broader immune mechanisms beyond cytokines driving viral pneumonia in humans.



ST23 - 173

Despite strong neutrophil induction, *Helicobacter pylori* escapes Neutrophil Extracellular Traps by degrading them

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Helicobacter pylori (*Hp*) is a pathogen colonizing the human gastric mucosa during early childhood, establishing a life-long infection through adulthood and importantly remains to this day the primary risk factor for the development of gastric adenocarcinoma. Although strong neutrophil infiltration into the lamina propria is the hallmark of *Hp* infection and considering their capacity to exert a multitude of antimicrobial responses, neutrophils fail to clear the infection and their specific role in the bacterial-induced gastric pathophysiology remains poorly understood. We aimed to investigate how human peripheral blood neutrophils interact with *Hp* clinical isolates, as well as, laboratory-adapted strains *in vitro*, for their ability to phagocytose and release neutrophil extracellular traps (NETs), visualized by confocal and live microscopy, while ROS generation was detected by flow cytometry. We also assessed the effects of both neutrophils and NETs on bacterial viability in solid cultures. Our findings demonstrate that neutrophils can phagocytose all *Hp* strains regardless of opsonization state and produce ROS regardless of the CagA expression status, or the number of EPIYA-C motifs. Interestingly, while NET release was documented in response to *Hp* infection, these NETs appeared degraded and demonstrated only faint levels of NET-associated proteins, compared to those induced by *E. coli* or mitogenic stimulation by ionomycin. Degradation of NETs was further verified via live microscopy and a myeloperoxidase (MPO)/DNA complex ELISA. Moreover, we detected presence of MMP-9 positive NETs in the gastric biopsies from *Hp*-infected individuals. Finally, we observed no significant effects in the bacterial growth and viability following incubation of *Hp* with neutrophils and NETs. Our findings suggest that while *Hp* can elicit a robust neutrophil response, the complex interplay between the bacteria and NETs may represent a new immune evasion mechanism that allows for bacterial life-long persistence.



ST24 - 4

Decoding Sulfated Hyaluronan–Mediated Changes in Matrix Composition and Functional Properties in 3D Cancer Models

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The extracellular matrix (ECM) plays a pivotal role in tumor development by regulating proliferation, invasion, and therapy response [1]. Hyaluronan (HA), a key ECM component, promotes tumor progression through effects on cell adhesion, motility, and growth factor signaling [2,3]. Chemically modified derivatives such as sulfated HA have been suggested to disrupt HA–receptor interactions and thereby influence malignant behavior. Importantly, sulfated HA shows reduced recognition and degradation by hyaluronidases (HYALs), enhancing its stability in the tumor microenvironment and potentially prolonging its modulatory effects. While two-dimensional (2D) cultures remain widely used, three-dimensional (3D) spheroid models more accurately reproduce tumor architecture and ECM interactions, providing a physiologically relevant platform for preclinical studies [4]. In our laboratory, we have already observed distinct treatment responses in 3D breast cancer models compared to conventional 2D cultures [5,6].

In this study, we investigated the effects of low-molecular-weight HA (50 kDa) and sulfated HA on MDA-MB-231 and MCF-7 breast cancer cell lines under both 2D and 3D conditions. Gene expression analysis of ECM-related and tumor progression markers, along with spheroid growth assessment and other functional assays were performed to compare cellular responses across models. Preliminary results show that both HA derivatives modulate gene expression, with sulfated HA producing more pronounced effects. Furthermore, differences between 2D and 3D cultures underscore the importance of model selection when evaluating ECM–tumor interactions.

Together, these findings suggest that sulfated HA may act as a promising modulator of breast cancer progression and highlight the relevance of 3D spheroid models for translational cancer research.

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ST25 - 269

Telomere specific tumor histopathology: a novel diagnostic tool for homologous recombination deficient ovarian cancers

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Inherited or acquired deficiency of homology mediated DNA repair (HRD) is currently therapeutically targeted in considerable subsets of breast, ovarian and prostate cancers. In the context of modern precision medicine, tumor HRD is clinically assessed in formalin fixed, paraffin embedded (FFPE) tissues, via Next Generation Sequencing (NGS) that identifies mutations in genes involved in homology-driven DNA repair and/or a series of genomic instability phenotypes representing the so-called genomic scars. Telomeres are particularly vulnerable to all types of DNA insults including oncogene-induced replication stress and in cancer they frequently acquire Double Strand Breaks (DSB) attracting recombinatorial DNA damage responses. Near 100% of human ovarian tumors sustain continuous neoplastic cell growth by activating the reverse transcriptase telomerase. It is now well established that telomerase is recruited to telomeres as damage response to DNA DSB. Hence, telomeres of HRD+ tumors are expected to become highly enriched in DSB and thus they may become hyper-elongated by telomerase. We tested this hypothesis in a telomerase positive cell line with normal expression of the major Homologous Recombination DNA repair component BRCA2, compared to isogenic BRCA2 knockout cells. We validated our pre-clinical experimental data in DNA extracted from a cohort of formalin fixed paraffin embedded (FFPE) tissue-samples from 50 human ovarian adenocarcinomas that were examined and categorized by NGS as HRD positive or negative. Histopathological FFPE specimens from this group were subsequently stained by dual-color telomere strand-specific fluorescence in situ hybridization. Deconvolved images were captured by Fluorescent Microscopy and/or by Multiphoton Microscopy and analyzed via specialized software tools. Our combined efforts expand our understanding on the involvement of DNA repair mechanisms in human malignancy, and most importantly they introduce novel, accurate, and broadly accessible diagnostic and prognostic approaches of HRD based on telomeric molecular pathology.



ST26 - 62

Integrative -Omics for Construction of Gene Regulatory Networks towards NKT Cell Identity Understanding

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Natural Killer T (NKT) cells are an oligoclonal T cell population that acquires life-long effector functions during a step-wise thymic differentiation process, without prior encounter with foreign antigen. Their maturation involves transitioning from progenitor to mature cells, culminating in the differentiation of three distinct NKT cell subsets. Although there are minor variations among NKT subsets, complex interactions between transcriptional and epigenetic regulators control their differentiation. Specifically, lineage-determining transcription factors (TFs) control unique gene expression programs by directly binding to accessible cis-regulatory elements at the genomic loci of their target genes. Here, we constructed a multi-omics bioinformatics framework integrating single-cell transcriptomic and epigenomic data to capture these interactions in the form of gene regulatory networks (GRNs) and reveal regulatory mechanisms that guide cell differentiation.

To begin to untangle NKT cell development, we purified thymic NKT cells through flow cytometry cell sorting and performed scRNA and scATAC sequencing. To explore developmental relationships between cell types, based on our scRNA-seq data, we generated pseudotime trajectories, which allowed the identification of transient cell states. In parallel, our scATAC-seq analysis identified distinct chromatin accessibility landscapes and uncovered accessible TF binding motifs. Integration of scRNA-seq and scATAC-seq data revealed TF regulators that influence NKT cell differentiation. Integration of accessible TF binding motifs with the expression and epigenetic patterns of the corresponding transcription factors and their putative target genes led to the construction of gene regulatory networks. Our succeeding goal is to project these GRNs across pseudotime to identify key regulons that are dynamically regulated throughout differentiation.

Our approach provides a framework for analyzing GRNs during NKT cell development, leading to an in-depth comprehension of NKT lineage commitment and diversification. With implications for immunology, developmental biology, bioinformatics, and possible therapeutic applications that target NKT-mediated immune responses, these findings advance our understanding of cell identity.

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ST27 - 128

Dynamic links between the cardiac intermediate filaments of the cytoskeleton and nucleoskeleton for proper cardiomyocyte differentiation, maturation and reprogramming.

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Accumulating evidence suggests that mechanical forces are integral to cardiac stem cell commitment and differentiation. The cytoskeleton—and specifically the desmin (DES) intermediate filament network and its association with nuclear lamins through the LINC complex—links the contractile apparatus to both extracellular and nuclear matrices, regulating trafficking and mechanotransduction throughout development and adulthood, governing myocyte homeostasis. Yet, DES' function in cardiomyocyte lineage differentiation and nuclear architecture remain insufficiently characterized.

In this work, we delineate the temporal and mechanistic involvement of DES in cardiac progenitor cell (CPC) specification, cardiomyocyte (CM) differentiation and maturation, and chromatin architecture across embryonic development, postnatal growth, and adult stress adaptation.

Using murine embryonic hearts, we first identify robust DES expression in CPCs as early as E7.5. Genetic ablation of DES markedly diminishes the proportion of PDGFRα^{+}/GFRα2^{+}/KDR^{+} CPCs, indicating that DES is required for maintaining progenitor identity and facilitating early cardiogenic lineage commitment. Furthermore, in direct reprogramming assays converting fibroblasts to induced cardiomyocytes (iCMs) via Gata4/Mef2c/Tbx5 (GMT) transcription factors, ectopic DES expression enhances reprogramming efficiency by approximately 50%, whereas DES deficiency results in impaired acquisition of mature CM characteristics, including myofibrillar assemply, calcium handling, and appropriate localization of cardiac specific proteins.

To investigate DES-mediated nuclear and epigenomic effects, we integrated RNA-seq, ChIP-seq, and Hi-C chromatin conformation analyses. Loss of DES disrupts higher-order chromatin organization and perturbs epigenomic organization and cardiac gene regulatory networks, establishing a direct link between cytoskeletal architecture and nuclear structural integrity. Collectively, our data establish DES as a multifaceted regulator in cardiac development: guiding progenitor cell dynamics, promoting CM fate and maturation, and maintaining nuclear/chromatin integrity — especially under stress. These findings redefine DES from a structural scaffold to a mechanotransduction player connecting the extracellular matrix to the nuclear lamina, thereby modulating genome architecture and cardiac-specific gene expression. Such studies offer mechanistic insights into desminopathies and identify potential targets for cardiac regenerative therapies.



ST28 - 102

T-box-i is a novel specific inhibitor of essential bacterial regulatory RNAs

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The emergence of pathogenic bacteria resistant to available mainstream antibiotics poses a major threat to public health worldwide, and particularly in Greece. Recent insights into regulatory non-coding RNAs in pathogens have gained attention for the development of a new generation of species-specific antibacterials. Among these, T-box riboswitches are RNA elements in Gram-positive bacteria that sense amino acid availability by detecting the aminoacylation status of cognate tRNAs, thereby regulating genes involved in amino acid transport and metabolism. Herein, we describe the characterization of T-box-i, a novel and specific inhibitor that suppresses *Staphylococcus aureus* planktonic cells and biofilm formation by disrupting T-box-mediated transcription *in vivo*. T-box-i was identified through a virtual screening campaign of commercially available small molecules against available crystallographic structures of T-box riboswitches. Importantly, T-box-i showed a low propensity for the development of resistance and did not exhibit any cytotoxicity in mammalian cells. In addition, RNA-seq analysis revealed distinct differentially expressed gene (DEG) profiles consistent with the inhibition of T-box–controlled operons and amino acid metabolism pathways. These transcriptomic changes validate the RNA-targeted mode of action of T-box-i and highlight its precision in modulating riboswitch-dependent gene networks. Collectively, these findings establish T-box riboswitches as promising antibiotic targets and underscore the therapeutic potential of RNA-targeted small molecules to combat antibiotic-resistant pathogens.

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ST29 - 109

Study of the GHRHR Activation Mechanism via Molecular Dynamics Simulations

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The Growth Hormone Releasing Hormone Receptor (GHRHR) is a member of the class B1 G-protein-coupled receptor (GPCR) subfamily.[1] GHRHR is activated by its cognate 44-amino-acid peptide ligand, GHRH. GHRHR signaling has clinically relevant links to oncogenesis: GHRH/GHRHR and tumor-expressed splice variants (e.g., SV1) form local autocrine/paracrine growth circuits in a wide spectrum of human cancers, and GHRH antagonists have demonstrated antiproliferative effects in many experimental cancer models.[2]

GHRHR consists of a large N-terminal extracellular domain (ECD) that confers ligand specificity, a canonical seven-transmembrane (7TM) domain that mediates signal transduction, and an intracellular C-terminal (CT) tail. Upon ligand binding, the N-terminal portion of GHRH engages the ECD, followed by insertion into the 7TM core, initiating receptor activation[3] which involves hallmark rearrangements of the transmembrane bundle, including outward movement of TM6 to open the intracellular G-protein-binding site and complementary adjustments of TM1 and TM7 to stabilize the active state.[4]

Although structural information obtained to date has advanced our understanding of GHRHR, the detailed mechanism by which ECD engagement is transmitted through the 7TM domain to the intracellular G-protein interface remains unknown. Here, we employ molecular dynamics simulations of full-length GHRHR to gain atomistic insights of the activation mechanism of GHRHR. Our analyses indicate that distal domains, including the CT tail and ECD, contribute to receptor activation by forming transient interactions with intracellular loops and the 7TM core. These dynamic contacts appear to modulate the accessibility of the G-protein-binding site and influence the conformational equilibria of the receptor. Correlation patterns suggest that the CT and ECD exhibit anti-correlated motions relative to the 7TM bundle, highlighting their potential role as allosteric regulators of signaling. These findings provide new insights into the mechanistic interplay between distal regulatory elements and the core activation machinery of GHRHR and may guide the design of modulators of receptor activation.

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ST30 - 233

Glycome studies on engineered MDCK and human primary nasal and tracheal epithelial cells for H3N2 influenza virus

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Human H3N2 influenza A virus recognises N-linked glycan receptors with *N*-acetylneuraminic acid (NeuAc) residues linked α2-6 to a galactose (NeuAcα2-6 Gal), referred as human type receptors. Mutations in the human H3 haemagglutinin (HA) have affected its binding specificity towards three or more LacNAc (Galβ1-4GlcNAc) repeats, whereas they have lost the avidity to the short LacNAc N-linked glycans. In order to investigate the effect of poly-LacNAc structures for infection and production of recent H3N2 influenza viruses, we have engineered MDCK, SIAT and hCK cell lines overexpressing the B3GNT2 *N*-acetylglucosaminyltransferase (NExt), a key enzyme for the biosynthesis of extended N-linked glycans. We employed matrix-assisted laser desoption ionisation time of flight (MALDI-TOF MS) instrumentation for the glycome characterisation. The SIAT-NExt cell line exhibited the highest relative abundance of poly-LacNAc structures, and the highest binding to the H3 HA and susceptibility to infection by recent H3N2 virus strains. Also, given the importance of chicken eggs for the growth of human influenza virus, additional glycome analysis on allantoic and amniotic egg membranes indicated that chicken eggs might not be the ideal medium for influenza virus growth. We have also investigated the existence of such receptors on nasal and tracheal primary epithelial cells from human respiratory tract, and we identified putative N-linked glycan receptors. Then, based on the structural diversity of the human airway glycome characterized, we constructed a synthetic N-glycan library and we found that bi-antennary N-linked glycans with one or two α2-6-sialylated extended antennae were the predominant receptors of recent H3N2 viruses. Glycome analysis has shown that such receptors for H3N2 human influenza virus exist on tracheal epithelium, albeit at very low relative abundance, but are deficient in nasal epithelium. These data, combined with immunofluorescence and single-cell RNA sequencing analysis suggested that H3N2 viruses have developed a tropism for tracheal ciliated cells.



ST31 - 201

Loss of the mitochondrial i-tRF^{GluTTC} results to early-relapse and poor treatment outcome in childhood B-cell acute lymphoblastic leukemia

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Background and aims: Childhood acute lymphoblastic leukemia (chALL) is the most common malignancy in children, representing ~33% of all pediatric cancers. Although chALL management is considered as one of the success stories in clinical oncology, ~20% of patients experience chemotherapy resistance and relapse, highlighting the need for modern personalized prognostic tools. tRNA-derived fragments (tRFs) constitute a novel subgroup of small non-coding RNAs, derived from nuclear or mitochondrial tRNAs, and can regulate gene expression post-transcriptionally. Herein, we have investigated the clinical utility of the mitochondrial internal tRF^{GluTTC} (mt-i-tRF^{GluTTC}) for patients' treatment outcome.

Methods: Bone marrow samples from 135 chB-ALL patients at diagnosis and 44 age-matched healthy controls were analyzed. Patients treated according to ALL-BFM95 and ALL-IC BFM2009 protocols. Following extraction, total RNA was polyadenylated in 3'-end and reversed transcribed, while mt-i-tRF^{GluTTC} levels were quantified by *in-house* qPCR. Patients' median follow-up was 10.3 years, while patients' relapse (DFS/PFS) and mortality (OS) were used as clinical endpoints.

Results: mt-i-tRF^{GluTTC} levels were significantly downregulated in chB-ALL patients compared to healthy children ($p<0.001$), while ROC curve (AUC=0.678; 95%CI: 0.580-0.777; $p<0.001$) and logistic regression (OR=0.865; 95%CI: 0.755-0.991; $p=0.036$) confirmed the loss of mt-i-tRF^{GluTTC} in chB-ALL. Kaplan-Meier survival curves highlighted the poor outcome of the patients with lower mt-i-tRF^{GluTTC} levels, in terms of worse DFS ($p=0.044$), PFS ($p=0.031$) and OS ($p=0.020$). Moreover, Cox regression analysis confirmed the significant higher risk of the patients with loss of mt-i-tRF^{GluTTC} for short-term relapse (HR=4.023; 95%CI: 0.929-17.42; bootstrap $p=0.043$) and worse survival (HR=36.21; 95%CI: 0.211-622.4; bootstrap $p=0.001$), while multivariate Cox regression models confirmed the poor prognosis of chB-ALL patients independently of the clinically established disease markers (WBC, prednisone response, BM morphology, MRD, risk-group, age).

Conclusions: Loss of mt-i-tRF^{GluTTC} at chB-ALL diagnosis is associated with significantly higher risk for early-relapse and poor survival following treatment, supporting the risk-stratification and personalized treatment prognosis.



ST32 - 184

Prevalence of Genetic Polymorphisms in Patients with a History of Thrombophilia

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The present study aimed to investigate the prevalence of thrombophilia related genetic polymorphisms in a general population sample and to evaluate their association with thromboembolic events. **Patients – Materials and Methods:** For this study, data from 713 patients, who had been tested for thrombophilia screening, where analyzed. Firstly, the basic hemostasis panel was performed (PT sec, PT INR, aPTT, Fibronogen, D-Dimers) and after Protein C and S activity levels, antithrombin III, plasminogen, homocysteine, activated protein C resistance (APCR), lupus anticoagulant and anticardiolipin (ACA) and anti-β_2-glycoprotein I (β_2-GPI) antibodies where measured. In addition, molecular testing was performed for the investigation of FV Leiden, FII G20210A, MTHFR C677T and MTHFR A19298C mutations. All samples were stored at -80°C and the data was processed with SPSS 29.0. **Results:** The prevalence of the genetic variants, which were investigated, was15.2% for FV Leiden mutation, 15.0% for FII G20210A, 73% for MTHFR C677T and 71% for MTHFR A1298C. Carries for the FV Leiden mutation demonstrated twofold increased risk of thrombosis (x^2 = 7.075, p = 0.008, OR = 1.80) and threefold increased risk of arterial thrombosis(x^2 = 9.893, p = 0.002, OR = 2.94). Moreover, carriers of the MTHFR A1298C mutation demonstrated more than double odds of cardiovascular disease (x^2 = 6.107, p = 0.013, OR = 2.45). In terms of clinical history, 31% of participants reported thrombosis, 14% venous thrombosis, 12% ischemic stroke, 14% pulmonary embolism, and 3% hematological disorders. Miscarriage, arterial thrombosis, and cardiovascular disease were each reported by 6% of the population. **Conclusions:** The results of this study highlight the significance of thrombophilia screening as part of thrombosis risk assessment. It is recommended that further studies be conducted to validate the clinical utility of these molecular biomarkers in broader populations.



ST33 – 237

Extranuclear roles of NuRD complex proteins: Novel insights in cell cycle progression

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The Nucleosome Remodeling and Deacetylase (NuRD) complex is a central chromatin regulator essential for gene transcription, genome stability, and cell cycle progression in mammalian cells. NuRD combines nucleosome remodeling with histone deacetylase activities, the latter mediated predominantly by HDAC1 and HDAC2. While HDAC2 is classically recognized for nuclear deacetylation and transcriptional repression, emerging work demonstrates that its roles extend to critical cytoplasmic events during mitosis.

Cytoskeletal remodeling plays a central role in eukaryotic cell division, migration, and morphogenesis, as well as pathological processes like cancer metastasis. Stable microtubules, key to metastatic dissemination, are defined by α-tubulin acetylation. Our previous studies show that CHD4 identified as a RanGTP-regulated microtubule-associated protein localizes to spindle microtubules and is essential for mitotic spindle assembly, prompting a re-evaluation of NuRD's wider role in cytoskeletal dynamics. Here, by using a "knock-sideways" system to temporally isolate protein function, we can distinguish CHD4's direct mitotic activities from its interphase roles.

Emerging data further demonstrate that HDAC2 is dynamically excluded from chromatin during mitosis, relocating to the mitotic spindle at metaphase. Functional depletion of HDAC2 in HeLa Kyoto and MCF10A cells disrupts spindle integrity, raises abnormal mitotic phenotypes, increases pericentriolar material, and elevates ciliation frequency, indicating HDAC2's role in suppressing ciliogenesis and maintaining centrosome function. Investigation across breast cancer cell lines reveals a positive correlation between centriole number and malignancy, with HDAC2 persistently localizing to the mitotic spindle, particularly at spindle poles in more aggressive tumors.

Altogether, these findings position NuRD, through CHD4 and HDAC2, as an integrative axis connecting epigenetic machinery to cytoskeletal organization, mitotic fidelity, and cellular processes fundamental to tumor development and progression.



ST34 – 249

Antiviral Activity Evaluation of Newly Synthesized Photocatalytic Surfaces for Environmental Decontamination – APOGEION –

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Introduction: Global health pandemics have highlighted an increasing need for a clean and safe environment in workplaces and hospitals. New innovative photocatalysts with semiconductor content of TiO_2, g-C_3N_4 and Ag_3PO_4 are emerging as ideal candidates for a wide range of antimicrobial coatings. We evaluated the antiviral activity of these permanent coatings activated by visible or blue (450-495nm) light, using model viral systems, such as the bacteriophage Qβ, an analogue for Influenza viruses, and herpes simplex virus type-1 (HSV-1).

Methods: We assessed antiviral activity by applying ISO 18071:2016 and ISO 21702:2019 for the determination of bactericidal activity of bacteriophage Qβ in *E. coli* cultures, and Vero cell culture lysis following HSV-1 infection. The photocatalytic antiviral effect was evaluated following overnight illumination of the viral suspensions under indoor light (Qβ and HSV-1) and blue light (HSV-1) of constant intensity (1000 lux) on both coated- and non-coated glass surfaces with a combination of coating materials. The double layer agar and plaque assay methods were employed to determine viral titers for Qβ and HSV-1, respectively. Resulting antiviral effects were expressed as ΔV values and percentage reductions of the viral titer. qPCR-based molecular methods were developed for detection of both viruses.

Results: Among the tested, non-cytotoxic surfaces, the TiO_2-based coating demonstrated the highest antiviral efficiency against Qβ, followed by g-C_3N_4 as a standalone agent. Combinations of TiO_2 with g-C_3N_4 or Ag_3PO_4 did not always enhance efficacy, suggesting possible inhibitory interactions. For HSV-1, mild efficacy was observed primarily for the TiO_2/g-C_3N_4 coating and secondly by g-C_3N_4 alone. Under blue-light illumination, however, the latter exhibited superior antiviral activity compared to the composite formulation, demonstrating strong inhibition of HSV-1 early and late gene transcription.

Conclusions: Our findings support the potential of these visible/blue-light-activated photocatalytic surfaces for application in environments requiring high hygiene standards, albeit in a virus-specific manner.

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ST35 - 279

Applications of Artificial Intelligence and data analytics for identifying molecular markers in Alzheimer's Disease

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Alzheimer's disease (AD) remains a major challenge due to its complex pathology and limited early biomarkers. Advances in data analytics and artificial intelligence (AI) are enabling the identification of molecular signatures crucial for diagnosis and therapy. By integrating multi-omics datasets, AI models can analyze large-scale data to uncover key biomarkers, optimize protein targets and predict disease progression. Machine learning and deep learning, combined with explainable AI techniques, support accurate biomarker discovery and biological interpretation. AI-driven data analysis thus facilitates protein optimization, molecular marker identification and patient stratification, paving the way for early diagnosis and personalized interventions in AD.



ST36 – 289

Novel COMP–PMEPA1 Interaction Rewires SMAD Signaling to Induce EMT in Breast Cancer

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Epithelial-to-mesenchymal transition (EMT) is a pivotal process in cancer metastasis, often driven by cues from the tumor microenvironment. Cartilage Oligomeric Matrix Protein (COMP) expression in breast cancer has been associated with poor patient survival and enhanced metastatic potential, yet its mechanistic role in EMT remains unclear. In this study, we demonstrate that COMP promotes EMT in breast cancer through functional and signaling reprogramming. Breast cancer cells exposed to recombinant COMP or engineered to express it showed a marked reduction in the epithelial marker E-cadherin, accompanied by increased expression of mesenchymal markers Vimentin and Versican. These alterations were also evident in COMP-expressing xenograft tumors, confirming their in vivo relevance. Functionally, COMP enhanced migration and invasion of breast cancer cells through both autocrine and paracrine mechanisms mediated by its thrombospondin (TSP) and C-terminal domains. Proteomic screening identified Prostate Transmembrane Protein, Androgen Induced 1 (PMEPA1) as a novel COMP-binding partner that mediates its EMT-promoting effects primarily via the COMP TSP domain. Analysis of The Cancer Genome Atlas (TCGA) breast cancer cohort revealed a positive correlation between COMP and PMEPA1 expression. Mechanistically, COMP reprogrammed canonical TGFβ signaling by shifting SMAD activation from pSMAD2/3 to pSMAD1/5, a switch consistent with EMT induction. Collectively, these findings establish the COMP–PMEPA1 axis as a new driver of EMT in breast cancer and suggest that targeting this pathway could offer therapeutic benefit against metastatic disease.



ST37 - 290

Bioactive NO for the treatment of long chain fatty acids oxidation disorders

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Long-chain fatty acid oxidation disorders (LC-FAODs) are rare genetic diseases with different clinical phenotypes, including hypoketotic hypoglycemia, cardiomyopathy, myopathy and liver failure. Very long chain acyl-CoA dehydrogenase (VLCAD) deficiency is the most severe among LC-FAODs. Current treatments provide only symptomatic relief, leaving patients at risk to develop life-threatening symptoms. Therefore, there is an unmet need to develop and test the efficacy of therapeutics for LC-FAODs.
Herein we tested the innovative concept to correct cardiac specific VLCAD deficiency by pharmacological administration of bioactive NO. Crossing VLCAD$^{flx/flx}$ mice with mice expressing Cre recombinase driven by the α-myosin heavy chain (MHC) promoter, we generated the cVLCAD$^{+/-}$ mice. Our data document that partial deletion of VLCAD in the heart results in lower protein expression and enzymatic activity as well as diminished fatty acid oxidation (FAO) capacity. Mice displaya metabolic dysfunction and develop LV hypertrophy similarly to humans with VLCAD deficiency. Chronic administration of sodium nitrite into the drinking water augments NO signaling as it is documented by the elevated levels of NO metabolites in the circulation and the heart. At the same time, it does not impact VLCAD protein expression however, it restores enzymatic activity and FAO rate in the heart. Moreover, mice display normal metabolic phenotype and the absence of cardiac abnormalities and dysfunction. At molecular level, bioactive NO administration augments S-nitrosylation of key enzymes participating to FAO pathway in the heart.
Collectively, this preclinical study provides proof of concept evidence regarding the therapeutic potential of bioactive NO for LC-FAODs. It paves the way for future studies that will test novel compositions that augment NO signaling and rewire metabolism as therapeutic intervention for LC-FAODs.

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ST38 - 288

Breast cancer-derived extracellular vesicles in chemoresistance and their use in liquid biopsies as diagnostic/prognostic biomarkers

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Despite the recent advances in cancer treatment, triple-negative breast cancer remains of unmet clinical need, with chemotherapy being the standard-of-care. Although about 30-40% of patients respond to neoadjuvant chemotherapy (NACT), 60-70% of them with either not respond or eventually relapse, often leading to metastasis occurrence. Our recent work has revealed that taxane-based chemotherapeutic drugs induce alterations in the biogenesis pathways of cancer cell-derived extracellular vesicles, in breast cancer. The altered chemotherapy-induced EV secretion and/or composition can modulate the premetastatic niche formation. The identification of the main molecular mechanisms that regulate this process may provide new targets to improve response to chemotherapy and prevent post-therapy metastasis formation. Our recent work sheds light on the cancer cell intrinsic mechanisms that drive cancer cell plasticity programs in response to NACT, leading to enhanced EV release. Importantly, we provide evidence that these phenotypic responses can occur due to pre-existing or adaptive mechanisms to the treatment. Since, tumour-derived EVs are secreted in the blood, they represent an excellent tool for diagnostic purposes using liquid biopsies. The circulating EVs represent an amalgam of heterogeneous subpopulations derived from different cellular sources. We show that the cargo of tumour-derived EVs may reflect their cellular origin. These cell type-specific EVs can be detected in the plasma of tumour-bearing mice and therefore, specific EV signatures could serve as biomarkers to dissect the heterogeneity of the primary tumour composition and its response to the treatment.



ST39 - 48

Chronic psychological stress and exosomes in precipitation and diagnosis of Alzheimer's disease brain pathology

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In the new era of Precision Medicine, the diagnosis, prognosis and treatment of complex, multifactorial brain diseases such as Alzheimer's disease (AD) and depression are increasingly based on the evaluation of the individual's lifestyle, risk factors and multilevel biological analyses that aim to clarify the degree of brain pathology as well as the effectiveness of new therapeutic schemes in each, individual/patient. Thus, the identification and usage of better biomarkers of brain health status are of paramount importance. Emerging evidence suggests extracellular vesicles (EVs), and specifically exosomes which are secreted membranous structures by cells and carry different biological material of the cell of origin, as an emerging "tool" of liquid brain biopsy with promising diagnostic value. Dr Ioannis Sotiropoulos will present clinical and experimental evidence about dual role of brain exosomes as disease propagators in the AD brain as well as carriers of biomarkers of brain pathology and will discuss the mediating role of brain EVs in the precipitating impact of chronic, psychological stress, a risk factor for AD, on AD-related Tau brain pathology. Given that the modern lifestyle increasingly exposes individuals to high stress loads, it is clear that understanding the mechanistic link(s) and the biomarker footprint of chronic stress in AD initiation and progression may facilitate the treatment of AD and of other stress-related disorders e.g. depression.



ST40 - 195

Prediction of Hepatic Steatosis in MASLD Patients Using Extracellular Vesicles: Shallow Deep Neural Networks Outperform a Foundation Model

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Background: Metabolic dysfunction–associated steatotic liver disease (MASLD) is the most prevalent chronic liver disease and strongly associated with obesity and cardiometabolic risk. Due to limited reliable noninvasive biomarkers, extracellular vesicles (EVs) have emerged as promising alternatives. Building on prior findings, we evaluated whether circulating EV characteristics can discriminate steatosis severity in an independent cohort [1-3].

Methods: In this retrospective multicenter study, 96 patients with metabolic dysfunction underwent steatosis assessment via transient elastography (iLivTouch FT100, Hisky). Steatosis stage (S0-S3) was defined using the Ultrasound Attenuation Parameter. EVs were isolated from fasting plasma by microfiltration after pre-clearing (3000 g, 20 min, 4 °C) and characterized using Nanoparticle Tracking Analysis (NanoSight NS300). Metrics included mean particle size, total EV count (50-1000 nm), and subpopulations (50-150 nm and >150 nm). Pearson correlation analysis showed a moderate association between EV counts and steatosis severity ($r \approx 0.35$). The cohort was split into training/validation (n=58; 3-fold CV) and external validation (n=38). Three models were evaluated: CatBoost, a shallow deep neural network (s-DNN; 3 layers: 13-5-7 neurons; ReLU; LR=0.01; 335 iterations; 'lbfgs' optimizer) [4-7], and TabICL. Performance for binary classification (S0 vs. S1-S3) was assessed using probabilistic ROC-AUC (rocp), sensitivity, and specificity.

Results: Both CatBoost and s-DNN generalized well. The s-DNN demonstrated stable performance across internal and external evaluation (rocp=0.75 both sets). CatBoost performed similarly (0.73±0.05 CV; 0.69 test) with balanced sensitivity (0.67) and specificity (0.69). TabICL underperformed and exhibited class-collapse behavior (rocp=0.54±0.08 CV; 0.65 test; sensitivity=0.11; specificity=0.90).

Conclusion: EV-based machine learning models, particularly lightweight architectures such as s-DNN and CatBoost, successfully distinguished absence of significant steatosis. Foundational models underfit small heterogeneous datasets, underscoring the importance of tailored, data-efficient approaches. These results support EV-driven AI as a minimally invasive strategy for early MASLD detection and precision hepatology.



ST41 - 297

An interpretable machine learning-based approach reveals patterns in extracellular vesicle formation and predicts transfusion efficacy in transfusion-dependent thalassaemia

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Extracellular vesicles (EVs), particularly small EVs (sEVs), are highly sensitive responders to cellular stress, reflecting dynamic pathophysiological states across numerous diseases. Their responsiveness makes them attractive candidates for early diagnostic and prognostic applications. Based on this premise, the information carried by EVs could be especially valuable when integrated into machine learning methodologies, enabling better exploitation of their complexity. Nanoparticle tracking analysis (NTA), remains the gold-standard method for quantifying EV concentration and diameter distribution in biofluids, yet, its conventional output metrics—mean size and concentration—capture only limited information. This study aimed to develop a more informative analytical approach for NTA data, capable of providing a richer description of vesicle distribution patterns and greater diagnostic resolution. This approach was applied to plasma sEVs from 16 transfusion-dependent thalassemia (TDT) patients and 17 healthy individuals, sampled before transfusion, 15–30 minutes after, and one week later, along with the supernatants of the transfused red blood cell (RBC) units. sEVs were isolated by chemical precipitation followed by filtration and characterized by NTA. A newly developed, machine-learning–compatible centroid approach was implemented capturing the 2D center of mass of each diameter probability distribution, thereby generating a morphometric index of the vesicle population with minimum information loss. Centroid analysis achieved superior discrimination between patients and controls compared with traditional NTA parameters and was sufficient to act as a single discriminant of these groups. Patients exhibited normal sEV concentrations but larger vesicle diameters. Post-transfusion distributions shifted toward those of controls, indicating partial restoration of vesiculation homeostasis. RBC units displayed diameter topologies similar to controls. Finally, the methodology was applied to myelodysplastic syndromes and hereditary spherocytosis to assess its broader utility. This proof-of-concept study introduces centroid-based analysis as a highly informative and biologically meaningful framework for EV characterization, and a promising non-invasive biomarker tool with direct clinical potential.

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ST42 – 57

PROTEOLYTIC ACTIVITIES OF EXTRACELLULAR VESICLES ATTENUATE A-SYNUCLEIN AGGREGATION

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Extracellular vesicles (EVs) are nano-sized lipid vesicles released into the extracellular space. We investigated the role of mouse brain-derived EVs in α-synuclein (α-syn) degradation and pathology transmission. Using sucrose gradient isolation and biochemical characterization, we found that EVs harbor active proteases that cleave both monomeric α-syn and pre-formed fibrils (PFFs). Protease activity and inhibitor profiling identified cathepsins B and S as key enzymes mediating this cleavage. EV-mediated proteolysis reduced the seeding capacity of α-syn PFFs in vitro and in vivo, whereas protease inhibition enhanced aggregation. Proteomic analysis revealed a restricted protease repertoire within EV cargo. Our findings suggest that EVs regulate extracellular α-syn levels via proteolysis, thereby modulating its prion-like spreading potential. We suggest that EVs represent a novel post-translational mechanism to regulate the levels of extracellular α-syn and may thus affect the spreading of α-syn pathology. Targeting this proteolytic capacity may offer new therapeutic interventions for mitigating synucleinopathies.



ST43 - 118

Structural and Functional Characterization of Alzheimer's Disease-Protective Apolipoprotein E Variants

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Apolipoprotein E (apoE), a major protein for lipid transport in circulation and the brain, has three common isoforms, apoE2, apoE3 and apoE4. *APOE4* is the strongest genetic risk factor for late-onset Alzheimer's disease (AD). Recently identified rare apoE variants, the apoE3(R136S)-Christchurch, apoE3(V236E)-Jacksonville and apoE4(R251G), appear to exert protective functions against AD and reduce the disease risk, but the molecular basis behind these effects is unknown. ApoE is a structurally dynamic protein, undergoing significant rearrangements that are important for its biological function. To examine the structural basis behind the properties of the protective apoE variants, we analyzed their structural and thermodynamic integrity both in *APOE3* and *APOE4* allelic backgrounds compared to their wild-type counterparts. Circular dichroism spectroscopy showed that only the V236E variation significantly alters the secondary structure of apoE3 and apoE4 in lipid-free form. This variant was also less prone to oligomerization. Chemical denaturation analysis indicated changes in the unfolding profile of V236E and R251G apoE variants in lipid-free form. Thermal unfolding analysis revealed small thermodynamic alterations in each variant compared to their wild-type apoE counterparts in lipid-free form, but a thermodynamic stabilization in lipoprotein-associated form. Additionally, following lipidation, all protective apoE variants were found to enhance the viability of SK-N-SH neuroblastoma cells and reduce the production of TNFα from BV2 microglia cells. Overall, these findings suggest that the specific amino acid changes found in AD-protective apoE variants can induce changes in the molecule's stability and conformation that may underlie common functional consequences, which are independent of the apoE background.

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ST44 - 15

Establishment of Patient-derived xenograft (PDX) models in the University of Patras: A tool for real time and 3-D cancer research

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Background: Patient-derived xenografts (PDX) represent a valuable tool for translational cancer research, as they retain the histopathological and genetic hallmarks of the original patient tumors. In the present study, we delineate the methodologies employed for the establishment of severe-combined immunodeficient (SCID) mouse colony and the development of two PDX models from patients with aggressive prostate cancer.

Methods: Animal facility conditions were optimised to support the rigorous requirements for maintaining a C.B.17 SCID mouse colony, encompassing specialised infrastructure for housing, routine sanitation procedures, and tailored nutritional protocols. Upon arrival of SCID breeders and after the mandatory quarantine time, a SCID colony of 25 mice was established. Tumor specimens from patients with aggressive prostate cancer were received from MD Anderson Cancer Center, The University of Texas, USA, and surgical implantation was performed subcutaneously in SCID male mice. Established PDX models underwent systematic drug administration including chemotherapeutic and epigenetic agents by intraperitoneal injections. At the termination of the experimental period, the harvested tumours were subjected to comprehensive morphological and molecular analyses, thereby enabling an in-depth characterisation of the established patient-derived xenograft models.

Results: Since April 2023, 240 breedings were performed, resulting in 191 successful births. Two PDX models of aggressive prostate cancer were successfully established. The PDXs' tumors displayed morphological and immunophenotypic features identical to those of the corresponding patient tumors. Drug administration led to a significant reduction in tumor size or growth rate, without any significant changes in tumor morphology.

Conclusions: In collaboration with the world's No.1 leading institution in cancer research, we have successfully established two PDX models within our institutional animal facility. To best of our knowledge, this represents the first tumor-derived PDX model developed in Greece. Our group also holds two of the three nationally approved PDX protocols (NTS-EL-969301, NTS-EL-515676), underscoring our leading role in preclinical prostate cancer research.



ST45 - 39

Hypomethylation in *A. thaliana* driven by a defense inducer is linked to salicylic acid-related expression upregulation

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Plants and their pathogens are evolving together. Pathogens manipulate host machinery to suppress defense responses, while plants deploy countermeasures to maintain immune resilience. In the Green Deal era, a safe and environmentally sustainable approach to supporting plants against pathogens is the use of plant defense inducers (PDIs), compounds activating defense and priming plants against imminent pathogens attacks. Adjustments of DNA methylation that activate gene expression are a key action by which PDIs induce plant defense. Here, chitosan nanoparticles loaded with salicylic acid (SA-CNPs), a previously studied PDI, influenced hypomethylation on specific genomic regions (Differentially Methylated Regions-DMRs) that corresponded to defense-related genes, such as pectin lyases, defensins and leucine-rich repeat transmembrane protein kinases. The most hypomethylated DMRs were the promoter and gene region of a lyase that degrades pectin from plant cell wall to induce cell apoptosis and notably, the promoter region of *SKP1A*, a core member of the SCF E3 ubiquitin ligase complex. Examination of both DMRs revealed the presence of salicylic acid-, auxin-, abscisic acid-, elicitor-, methyl jasmonate-, and defense-related cis-elements. Investigation of proteins associated with the above cis-elements revealed significant upregulation after SA-CNPs application. Moreover, correlation of the identified DMRs with transcriptomics, produced under the same experimental conditions, showed 43 genes that shared both groups. Among these genes THI2.1 (THIONIN2.1), GRP-3 (GLYCINE-RICH PROTEIN 3), EDS5 (ENHANCED DISEASE SUSCEPTIBILITY 5), PXMT1 (1,7-PARAXANTHINE METHYLTRANSFERASE) and CRK34 (CYSTEINE-RICH RECEPTOR-LIKE PROTEIN KINASE were associated with the 'response to salicylic acid' Gene Ontology (GO) term. Functional protein association network analysis in the STRING platform revealed association of SKP1A with COI1 (CORONATINE-INSENSITIVE PROTEIN 1) a primary interactor of THI2.1. Overall, these findings shed light on the epigenetic mechanisms that underly salicylic acid- related defense priming in plants.



ST46 - 45

ERAP1 Allotypes 2 and 10 Differentially Regulate the Immunopeptidome of Melanocytes

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Endoplasmic reticulum aminopeptidase 1 (ERAP1) is a polymorphic intracellular enzyme responsible for trimming peptides within the endoplasmic reticulum, thereby shaping the cellular immunopeptidome. In humans, at least ten common allotypes are known and display distinct enzymatic activities, which influence antigen processing. Polymorphic variation in ERAP1 has been linked with susceptibility to several HLA-associated autoimmune diseases, including Ankylosing Spondylitis, Uveitis, and Psoriasis, as well as cancer. These associations are believed to result from the generation of distinct $CD8^+$ T cell repertoires recognizing HLA-bound peptides. Given the broad impact of ERAP1 activity on antigen processing, we hypothesized that allotypic variation may cause notable shifts in the immunopeptidome, thereby influencing antigen presentation and immune recognition. To test this hypothesis, we generated stably transfected melanoma cell lines expressing ERAP1 allotypes 2 and 10—variants that differ in catalytic activity—under interferon γ stimulation. Comparative analysis of the immunopeptidomes derived from these two clones revealed extensive quantitative differences in peptide presentation, encompassing variations in length distribution, sequence motifs, and predicted binding affinities. Such alterations can profoundly impact immune receptor recognition. Taken together, these findings indicate that the functional enzymatic diversity of ERAP1 allotypes directly shapes the cellular immunopeptidome, offering mechanistic insight into how ERAP1-dependent antigen processing contributes to HLA-associated autoimmune conditions and tumor immunology.

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ST47 - 232

"When Less becomes More": Ultra-sensitive proteomics applications down to the single cell level

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Ultra-sensitive proteomics and single-cell proteomics (SCP) are reshaping the frontier of protein analysis by enabling detailed molecular insights from minimal biological material, down to the picogram level. Ultra-sensitive proteomics leverages cutting-edge mass spectrometry to analyze complex biological samples that were previously challenging due to limited amount/quantity. Together with SCP, which characterizes the proteome of individual cells, these approaches overcome the limitations of bulk analysis, allowing precise profiling of proteome heterogeneity. This enhanced resolution paves the way for a deeper understanding of cellular functions and disease mechanisms.

Our facility has recently acquired state-of-the-art mass spectrometry equipment, single-cell isolation instrumentation and advanced data analysis software through the EU-funded Recovery & Resilience Fund. This has enabled us to apply various methodologies that encompass single-cell proteomics, epiproteomics, immunopeptidomics, secretomics, extracellular vesicle proteomics and spatial proteomics. Each of them is addressing different biological questions that can be applied to different samples, including biofluids such as plasma, follicular fluids and tears, as well as material originating from tissue macrodissections, such as cancer biopsies. Together, these methodologies deliver unparalleled molecular detail that accelerates discoveries in biomarker identification and can thus enable the development of personalized diagnostics and therapies.

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ST48 - 240

Developing Metabolomic Tools for Assessing Perinatal Exposure to Endocrine-Disrupting Pesticides

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Pesticides are suspected as endocrine disruptors (EDs), yet assessing their impact on human physiology remains challenging. Population exposomics, particularly when combined with metabolomics, offers a promising framework for understanding how environmental factors such as EDs influence health. However, the field still lacks standardized protocols regarding study design, cohort definition, analytical approaches tailored to specific compounds and their metabolic pathways in humans and thus extensive human data to support concrete conclusions. Main objective of this study is to explore perinatal exposure to pesticides in Thessaly, the main agricultural region of Greece, using metabolic profiling analysis. The study focused on the identification of active ingredients (deltamethrin, dimethomorph, chlorpyrifos, 2,4-D, flupiradifurone, cyprodinil/fludioxonil, abamectin) in seven commonly used in the region pesticides, in the blood serum of pregnant women and their male offspring (umbilical cord samples) to be correlated with demographic and biomedical data.

As most of the active ingredients have not been extensively studied, we had to establish LC-MS based protocols to identify the detection limit for each compound, as their serum concentration may vary depending on the type and duration of exposure, and to enable the collective detection of the pesticides and the metabolic profile of each individual. We also collected knowledge about the compounds, their physicochemical properties, potential ways of exposure to them and their introduction to the body along with molecular functions that could be disturbed. Finally, serum samples from four mother–newborn pairs were analyzed in this preliminary phase. Active ingredients of three pesticides (chlorpyrifos, cyprodinil/fludioxonil, and abamectin) were identified in both maternal and cord blood, suggesting placental transfer. Chlorpyrifos was not detected in one participant, consistent with her urban residence and nonagricultural occupation.

Detectable residues in serum support the extension to larger cohorts, in order to reveal links between prenatal environmental exposure and endocrine outcomes.



ST49 - 286

Identifying drivers of clonal expansion in esophageal epithelium through a CRISPR-Cas9 competition screen

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Human epithelial tissues are colonized by somatic mutations, which are subjected to selection influenced by intrinsic and extrinsic factors. Recent studies indicate that by middle-age, cancer-driver mutations prevail the human epithelia, yet tumour incidence remains rare. Extensive analysis of newly-formed, microscopic, tumours in a mouse model of esophageal carcinogenesis revealed that pre-malignant tumours are eliminated through competition with adjacent mutant clones of the normal epithelium[1]. Thus, the dynamic competition between mutant clones serves as an anti-tumorigenic mechanism in normal epithelia. Here, we describe a novel, self-sustained, 3D culture system of human and mouse epithelia, which allows long-term studies of clonal expansion and competition. Epithelioids, acquire the physiological architecture of epithelial tissues and are maintained in a homeostatic state that does not require cell passaging. Proliferative basal cells gradually differentiate and migrate to suprabasal layers. Keratinised differentiated cells lay in the apical side of the culture, and they eventually shed, similar to physiological epithelial21 tissues. Epithelioids support genetic manipulations and successfully replicate *in vivo* cell dynamics. Aiming to investigate the complex interactions of mutant clones in normal esophageal epithelium, we performed a CRISPR-Cas9 screen in mouse epithelioids. A targeted gRNAs library was designed based on the expression profile of esophageal epithelial cells. Mutant and control cells were allowed to "compete" in the three-dimensional system of epithelioids and subsequent targeted sequencing revealed the differences in gRNAs abundance. Ultimately, identifies novel drivers of clonal expansion resolving the genetic networks underpinning competitive fitness and highlight epithelioids as a versatile culture system.

1 B. Colom et al., Mutant clones in normal epithelium outcompete and eliminate emerging tumours. Nature 2021



ST50 - 127

Diet-responsive proteogenomic effects following short-term restriction of animal products in humans

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Most studies exploring the impact of regulatory genetic variants capture effects of steady-state environmental conditions. Understanding the complex interplay between environmental factors, such as dietary intake, and genetics has the potential to enrich our understanding of key mechanisms that contribute to higher level phenotypes, including disease. Here, we investigated gene-by-diet interactions in a unique group of apparently healthy individuals (N=200) who alternate between omnivory and dietary restriction of animal products for religious reasons and compared findings to a continuously omnivorous control group (N=211). Using genotype data and repeated plasma proteomic measurements, we employed state-of-the-art methods, such as quantitative trait loci (QTL) mapping, to associate genetic variants to protein levels across dietary groups and sampling time points. To explore potential downstream consequences of these genotype-dependent effects on proteins, we combined our results with published GWAS variants and performed colocalization analyses. This allowed us to assess whether the diet-responsive genetic effects could also influence other health-related traits. We identified diet-responsive cis-protein quantitative trait loci (cis-pQTLs) including gain of genetic regulatory effects for LBR and MSRA, proteins linked to cholesterol and methionine metabolism respectively. LBR-associated diet-responsive cis-pQTL rs74148404 showed suggestive evidence of colocalization with obesity exclusively under dietary restriction, suggesting diet-dependent differential genetic risk for disease. Our analyses also uncovered seasonally dynamic gain and loss of genetic regulation, unique to the control group, for proteins linked to apoptosis in immune system pathways (MAVS, CASP3, PDLIM7, IL12RB1), effects likely masked by animal product restriction. These findings reveal dynamic diet- and season-sensitive regulatory mechanisms with implications for precision nutrition and individualized disease prevention strategies, and underscore the need to integrate environmental context into genetic studies of health and disease.



ST51 - 110

Integrated omics and biomarker profiling reveal mechanisms of adverse transfusion reaction in a thalassemia patient

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Red blood cell (RBC) transfusion is a mainstay therapy for patients with transfusion-dependent thalassemia (TDT). However, it carries cumulative risks and may have adverse effects like allergic or febrile reactions. Acute pain transfusion reaction (APTR) is a rare entity of unknown etiology. Herein, we report an APTR event in an adult subject with TDT. Patient and returned RBC unit samples from APTR (T0), a preceding (T-1) and subsequent (T+1) transfusions were compared between each other and against reference groups of patients and RBC units with similar genetic profile and storage duration, respectively. Analyses included spectrofluorometry, electron microscopy, nanoparticle tracking, targeted metabolomics of RBCs and plasma, and DIA-NN proteomics analysis of RBC membranes, followed by statistics and bioinformatics. APTR was associated with acute redox imbalance, cell adhesiveness, and membrane-specific proteostatic failure. Multi-omics analyses revealed expression of the RANTES/DARC chemokine axis, and other biological response modifiers in patient RBCs and plasma, suggesting cell activation and nociceptive signaling. The implicated RBC unit exhibited storage lesions disproportionate to its age, including prominent spheroechinocytosis, elevated intracellular reactive oxygen species, phosphatidylserine exposure, excess of adenine and ophthalmate, but depletion of glutamate and carnitine. ICAM4, STEAP3, DARC, alpha-synuclein, and other proteins were overexpressed, linking RBC dysfunction to inflammation and pain sensitization. The APTR-associated pathologies only partially improved over two weeks, suggesting long-term effects related to proteostasis, and systemic hypercoagulability, as revealed, among other findings, by the binding of thrombospondin to RBCs. Together, the data supports a two-hit model in which patient risk factors and those conveyed by blood components interact to trigger APTR as an RBC-centered vascular and immunological crisis in the TDT milieu. They also highlight the active role of RBCs in processes beyond oxygen transport, including erythrocrine activity and systemic signaling, through binding, uptake, processing, and release of cytokines, metabolites and other BRMs.



ST52 - 143

Targeted epigenetic modulation of *ZBTB7A* by episomal CRISPR/dCas9 systems enhances *HBG* expression in K562 cells

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The transcription factor ZBTB7A (LRF) is a known repressor of γ-globin gene expression, cooperating with BCL11A and chromatin remodelers to silence fetal hemoglobin (HbF) in adults. Reactivation of HbF through modulation of γ-globin genes' repressor LRF could therefore represent an innovative strategy for β-hemoglobinopathy treatment. We designed episomal CRISPR systems to achieve sequence-specific epigenetic repression of *ZBTB7A* gene in K562 cells and allow for gene regulation without introducing permanent genomic alterations. The catalytically inactive Cas9 protein, dCas9, fused with either DNA methyltransferase 3A (*DNMT3A*), for targeted DNA methylation, or histone deacetylase 1 (*HDAC1*), for histone deacetylation, was incorporated within the pEPI-1 established episomal vector. The human erythroleukemia cell line K562 was co-transfected with both CRISPR-constructs along with specific guide (sg)RNAs targeting the CpG island 326 upstream of *ZBTB7A*, allowing the epigenetic enzymes to modulate the epigenetic landscape simultaneously. The pEPI-1 backbone includes the Scaffold/Matrix Attachment Region (S/MAR), to ensure mitotically stable, non-integrative maintenance and stable expression of the constructs. K562 transfected double-positive clones were continuously cultivated for 80 days and analyzed at three time-points. Digital PCR confirmed stable episome retention at 1,5-2 copies per cell throughout the duration of cell culture. qPCR revealed a 4-fold reduction in *ZBTB7A* expression, by the third time-point, accompanied by a moderate, but stable increase in methylation within the targeted CpG island, near the sgRNA targeting site, assessed by pyrosequencing CpG assay. Downregulation of *ZBTB7A* led to a 2-fold increase in *HBG* expression and enhanced HbF hemoglobin synthesis, verified by digital PCR and benzidine staining, respectively. This study demonstrates a locus-specific epigenetic intervention in *ZBTB7A* expression, using a stable, episomal CRISPR/dCas9 platform, and enables for the precise gene modulation without genomic integration, highlighting its potential as a therapeutic tool in β-hemoglobinopathies.



ST53 – 221

Mechanistic Insights into Transcription Dynamics Uncovered by In Silico Simulation and Mathematical Modeling

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Our research aims to better understand the complex and inherently stochastic molecular interactions that can nonetheless drive an extraordinarily robust biological network regulating RNA transcription levels. We attempted to model this process faithfully in silico and predict essential parameters driving the system. Here we present an implementation of a flexible and comprehensive in-silico transcription simulation tool – iTranscribeRNA – relying on mathematics models (MM) and machine learning (ML) strategies to understand principles of transcription dynamics in human cells in physiological or perturbed cellular environments. iTranscribeRNA can i) test RNA polymerase II (Pol 2) position, speed or density and nascent RNA (nRNA) topology and levels across genes and regulatory regions and ii) determine how Pol 2- ,epigenetic- , and RNA-associated reactions depend on each other and what combinations of molecular events determine gene expression output. A first key aspect was to faithfully simulate incremental progression of Pol 2 on DNA template strand of virtual genes as required by the biochemical nature of the reaction of nucleic acid polymerization from its binding to promoters to initiation, pausing and release into elongation, stalling, back-tracking, termination and recycling. A second effort involved the design of a Markov chain mathematical model that predicts with high accuracy the observed Pol2 initiation and release rates. This is instrumental to provide theoretical estimation of the transcription system and it enables us to interpret key biological network properties controlling individual genes' expression level (e.g. promoter binding, initation, rate, proximal-promoter pausing or release…). Finally, our tool can search for model's parameter values that better fit the simulated data to biological data. It means that it can be used to predict how the transcription system behaves in particular cell types, during development or under stress and disease conditions, highlighting features that will undoubtedly be valuable to reveal drugable targets for transcription-addicted cancers.

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P1 – 42

Selenium Binding Protein: Exploring a novel stress regulator in the model microalga *Chlamydomonas reinhardtii*

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Selenium-binding proteins (SBPs) represent a ubiquitous protein family implicated in various environmental stress responses, although the exact molecular and physiological roles of the SBP family remain elusive. In our work, we identified and characterized *Cr*SBD1, an SBP homolog from the model microalgae *Chlamydomonas reinhardtii*. Growth analysis of the *C. reinhardtii sbd1* mutant strain revealed that the absence of functional *Cr*SBD1 resulted in increased growth under mild H_2O_2-induced oxidative stress, although cell viability rapidly declined at higher concentrations. Combined global transcriptomic and metabolomic analyses indicated that the *sbd1* mutant exhibited a dramatic quenching of molecular and biochemical responses to stress compared to the wild type. Our results suggest that *Cr*SBD1 functions as a cellular regulator involved in modulating the early oxidative stress responses of *C. reinhardtii*. In addition, yeast two-hybrid assays indicate that *Cr*SBD1 is part of an extensive and conserved protein–protein interaction network that includes Cysteine endopeptidase 2 (*Cr*CEP2). To further elucidate the role of SBP and its interactome in stress sensing and response, we are currently working with a *Cr*CEP2 mutant to investigate whether CEP2 also participates in the oxidative stress response as a key component of this mechanism, and if the unresponsiveness observed in the *sbd1* mutant strain is solely due to the absence of SBP or involves broader interactions within this protein network.

The research project is implemented in the framework of H.F.R.I call "4th Call for H.F.R.I.'s Research Projects to Support Postdoctoral Researchers" (H.F.R.I. Project Number: 28950).



P2 - 52

Bacterial diversity analysis of glyphosate-exposed and pristine soil samples

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Glyphosate is the active ingredient in widely used broad-spectrum herbicides, and its extensive application raises increasing concerns about environmental accumulation and ecological impact. It is known to be toxic to aquatic organisms, while numerous studies have also reported adverse effects on plant growth, insect gut microbiota, and human health. Therefore, research on soil bacterial communities can significantly contribute to the discovery of novel and effective strategies for the bioremediation of glyphosate-contaminated sites.

In this study, metagenomic analysis based on 16S rRNA gene amplicons was performed on soil samples from an agricultural site with prolonged glyphosate exposure and a reference forest soil with no history of herbicide application. The bacterial composition of each sample was evaluated, revealing high overall microbial diversity. Actinobacteria and proteobacteria were the dominant phyla across all samples, while the relative abundance of other phyla varied depending on the sampling site. Marked differences were observed at the genus level, distinguishing the forest microbiome from those exposed to glyphosate. The presence of species and genera previously reported to degrade glyphosate was also examined. Functional profiling based on 16S rRNA amplicons predicted ABC transporters, glutathione S-transferases, and ECF RNA polymerase sigma-70 factors—proteins typically associated with stress response—as differentially abundant between samples.

These findings provide insight into how chronic glyphosate exposure shapes soil microbial communities and their potential functional adaptations, highlighting microbial diversity as a valuable resource for developing sustainable bioremediation strategies for glyphosate-polluted environments.



P3 - 81

Soil microbiota as affected by winter service crops in vineyards

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Service crops play a crucial role in agricultural systems by contributing to weed management and improving soil health. Through ground coverage, these plants compete with weeds for sunlight, water, and nutrients, thereby suppressing their growth and reducing the reliance on herbicides and mechanical weed control methods. This study presents findings from a one-year field trial conducted during the 2024 growing season, aimed at evaluating the effects of various winter-sown service crops under semi-arid Mediterranean conditions in vineyards. The trial took place in a non-irrigated vineyard located in Askri, Boeotia (Central Greece; coordinates: 38.314194, 23.111889), where service crops were sown on December 10, 2023. The experimental treatments included: T1 – *Lathyrus sativus*, T2 – *Pisum sativum*, T3 – *Hordeum vulgare*, T4 – *Trifolium subterraneum*, and T5 – a mix of *Hordeum vulgare* and *Vicia sativa*. Soil bacterial biodiversity was analyzed using the Illumina sequencing platform, with results detailing the composition and observed changes in soil bacterial communities. Bacterial community changes have been assessed, using 16sRNA data, by studying ecological indices and differential abundance responses. The analysis workflow revealed a temporal gradient in soil bacterial community composition. Across all treatments, a coherent successional change in community structure over time was observed. In addition, later time samples showed greater dispersion, reflecting divergent local responses to cover crop establishment and soil microenvironmental variability. Preliminary functional pathway analysis results highlighted that predicted carbohydrate degradation and energy metabolism pathways were the most dynamically enriched across timepoints. Pathways such as D-galacturonate degradation I/II, D-glucarate and D-galactarate degradation, and Enter-Doudoroff pathway exhibited strong time-dependent shifts.



P4 - 104

Emerging viruses and Virus-like Particles (VLPs): Developing surrogate systems for Pandemic Preparedness Countermeasures

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The recent COVID-19 pandemic coupled with the continuous threat of emerging viruses due to climate change and disturbance of ecological niches, has underlined the global need for accelerating research, improving surveillance, and efficiently developing medical countermeasures against pathogens with pandemic and epidemic potential, such as emerging RNA viruses SARS-CoV-2 and West Nile (WNV) or influenza A virus (IAV). VLPs are protein-based, nanoscale, self-assembling structures with many key applications in biomedicine, especially in the development of vaccines and drug carriers. Importantly, they facilitate new drug assessment and act as low biosafety level non-infectious viral proxies for the efficacy testing of novel *in vitro* diagnostic methods. This study aimed to produce SARS-CoV-2, WNV and IAV VLPs using a cell culture system and investigate their ability to promote cell responses. We used HEK-293 cells to generate VLPs by co-transfecting three sets of expression plasmids coding for the SARS-CoV-2, WNV and IAV structural proteins. Immunoblotting verified the presence of capsid proteins in purified VLP preparations following ultracentrifugation on sucrose gradients. Size and concentration of particles were evaluated by Nanoparticle tracking analysis (NTA). Functional analyses involved assessment of SH-SY5Y fully differentiated neurons' viability using SPARK-CYTO imaging. Successful generation of both VLP types was ascertained by the presence of the four SARS-CoV-2 structural proteins Spike (S), Envelope (E), Membrane (M) and Nucleocapsid (N), the WNV pre-membrane (prM) and envelope (E), the IAV haemagglutinin (HA), neuraminidase (NA) and Matrix-1 (M1) proteins. NTA revealed the presence of specific size peaks for VLP each species, For SARS-CoV-2 and WNV VLPs, we observed enhanced cell rounding, disappearance of neurites and cell death following incubation of cultures with VLPs, albeit, WNV exerted faster, more detrimental effects, in accordance with its known neurotropic actions. Our data demonstrated the generation, quality control and functional validation of VLPs for the emerging viruses in question.

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P5 - 122

Arabidopsis KUP/KT/HAK Transporters of Clade IIa Are Essential for Gametogenesis and Embryogenesis

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The Arabidopsis KUP/KT/HAK family includes thirteen members of evolutionary conserved high-affinity potassium transporters. Phylogenetic analysis placed TRH1/KUP4 and KUP3 within the same subgroup (Clade IIa). While TRH1/KUP4 potassium/ H^+ cotransporter is known to modulate auxin homeostasis in roots, affecting both root hair formation and gravitropic response, the biological function of KUP3 remains poorly characterized. Here, we investigate the potential functional interaction between TRH1/KUP4 and KUP3, given their strong sequence homology and the ability of TRH1/KUP4 to form homodimers. Biometric analysis showed that *kup3* single mutant exhibited wild-type-like root hair length and normal gravitropic responses. However, during the generation of a *trh1/kup4 kup3* double mutant, two intermediate genotypes were recovered: line 3.1 ($TRH1^{-}/^{-}$ $KUP3^{+}/^{-}$) and line 3.2 ($TRH1^{+}/^{-}$ $KUP3^{-}/^{-}$). Screening large progeny populations from both lines failed to yield any $TRH1^{-}/^{-}$ $KUP3^{-}/^{-}$ double mutant individuals, suggesting that simultaneous loss of both genes causes lethality. Segregation analysis of line 3.1 siliques revealed a 1:1 ratio of viable to aborted seeds, consistent with early embryo abortion caused by a $TRH1^{-}$/ $KUP3^{-}$ gamete (1n). In contrast, line 3.2 showed normal seed set without any gaps but a distorted segregation pattern: 86% of the offspring were *kup3* single mutants, while 14% retained the parental genotype ($TRH1^{+}/^{-}$ $KUP3^{-}/^{-}$). The complete absence of the $TRH1^{-}$/ $KUP3^{-}$ gamete (1n) that previously led to early embryogenesis abortion in line 3.1, suggests that this gamete type is non-viable in line 3.2. Moreover, the reduced frequency (14% instead of the expected 50%) indicates that even the reciprocal $TRH1^{-}$/ $KUP3^{-}$ gamete is severely compromised, resulting in few viable escapers and an excess of *kup3* single mutants. Taken together, these findings reveal a previously unknown synergistic relationship between the members of clade IIa KUP/KT/HAK potassium/ H^+ cotransporters in gametogenesis and early embryogenesis, suggesting a potential physical interaction between TRH1 and KUP3 subunits.

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P6 - 124

How Olive Fruits Make Their Oil: Developmental, Metabolic and Hormonal Control

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The health-promoting properties of olive oil originate from the unique profile of fatty acids (FA). However, the transcriptional mechanisms regulating FA biosynthesis during drupe ripening remain poorly understood. In this study, we integrated transcriptomics, targeted FA profiling and weighted gene co-expression network analysis (WGCNA) to elucidate lipid metabolism through four developmental stages of Olea europaea cv. 'Koroneiki' drupes. Fatty acid quantification revealed a gradual decline in saturated fatty acids (SFAs) accompanied by a steady increase in monounsaturated (MUFAs) and polyunsaturated fatty acids (PUFAs), particularly oleic and linoleic acids. Transcriptomic analysis identified 42 core enzymes involved in FA metabolism and triacylglycerol (TAG) biosynthesis. WGCNA uncovered distinct transcriptional modules associated with SFA reduction, late-stage MUFA accumulation and PUFA synthesis during ripening. Genes of the saturation pathway were progressively downregulated, while those of the desaturation pathway, which are known key determinants of final oil composition, were upregulated, resulting in oleic acid prevalence. Notably, ABA biosynthesis and signaling genes co-expressed with MUFA/PUFA modules, highlighting a pivotal role for ABA in late-stage lipid biosynthesis. Furthermore, ABA-mediated regulation of lipid metabolism appeared to be modulated by differential expression of specific *PP2C* homologs and coordinated by distinct transcription factors. The expression dynamics of *stearoyl-ACP desaturase* (*SAD4*) and the TAG assembly enzyme *PDAT1* mark the transition from saturated to unsaturated FA synthesis, resulting in ABA-mediated oleic acid enrichment. Overall, we present an integrated systems-level framework of the transcriptional networks driving olive oil biosynthesis, outlining a molecular toolbox to enhance extra virgin olive oil yield and quality.

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P7 - 251

Proteomic Profiling of Doxorubicin-Treated SK-N-SH Neuroblastoma Cells and Cytotoxic Evaluation of Cannabinoid Co-Treatment

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Neuroblastoma remains a challenging pediatric malignancy with limited therapeutic options and pronounced chemoresistance. Doxorubicin (DOX), a commonly used chemotherapeutic agent, exerts its cytotoxic effects through DNA intercalation and oxidative stress, yet its efficacy is constrained by dose-dependent toxicity. Cannabinoids have recently emerged as potential adjuvants with anti-tumor and cytoprotective properties. This study aimed to characterize proteomic alterations in the SK-N-SH neuroblastoma cell line following treatment with DOX, to elucidate key mechanisms underlying their potential synergistic effects.

Cell viability, assessed by the resazurin reduction assay, was used to determine the cytotoxic profile of DOX and cannabinoid treatments and to select optimal concentrations for subsequent proteomic analysis. The results revealed a dose-dependent decrease in metabolic activity, confirming the cytotoxic impact of DOX and informing the experimental conditions for proteomic profiling.

Label-free quantitative proteomics identified extensive remodeling of the SK-N-SH proteome upon DOX exposure. Principal Component Analysis (PCA) showed distinct clustering between treated and control samples, indicating pronounced proteomic divergence. The most upregulated proteins included PLG, APOE, GRSF1, and UBAC2—linked to coagulation, lipid metabolism, and RNA processing—reflecting stress-induced reprogramming of cellular functions. Conversely, EIF4B, RPL19, and PGAM1 were among the most downregulated proteins, implicating translational suppression, metabolic inhibition, and oxidative stress as hallmarks of DOX-induced cytotoxicity.

Pathway enrichment analysis highlighted activation of the complement and coagulation cascades, lipid particle remodeling, and interferon-stimulated oxidative stress responses, underscoring the interplay between inflammatory, metabolic, and apoptotic signaling. Concurrent downregulation of redox-regulating (GLRX, TXNDC17) and cytoskeletal (CFL1, ACTG1) proteins further emphasized mitochondrial and structural dysfunction during apoptosis. These results pave the way for further investigation of how cannabinoid co-treatment may influence these pathways, potentially reducing DOX-induced toxicity while enhancing anti-tumor efficacy.



P8 - 187

Dietary supplementation with lactic acid bacteria has beneficial effects against High fat diet induced obesity and DSS induced colitis in mice.

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The importance of lactic acid bacteria in health as constituents of traditional nutrition, including Greek traditional products, has been acknowledged and researched extensively, adding value to fermentation products or products containing probiotic strains. Lactic acid bacteria have a direct impact on gut microbiome composition, with profound effects especially on the immune system. However, the role of selected strains isolated in Greece, in the context of pathologic conditions, such as colitis and diabetes, remains elusive.

To address this, we utilized multiple strains of lactic acid bacteria as diet supplements in murine models of High fat diet (HFD) and DSS induced colitis. Specifically, C57BL/6 mice were fed with HFD supplemented with *Lactococcus cremonis* or *Lactococcus plantarum* for 8 weeks. HFD fed mice and lean diet fed mice were used as positive and negative control groups respectively. All groups were weighted on a weekly basis, and their blood glucose levels were evaluated via Glucose tolerance test. The results showed that diet supplementation with *Lactococcus plantarum* partially limits weight gain and reduces the glucose levels of HFD fed mice. Additionally, to evaluate the anti-inflammatory capacity of a *Pediococcus acidilactici* strain against colitis, we fed mice with the probiotic in chow diet and subsequently subjected them to DSS-induced colitis. We noticed that upon DSS administration, *P. acidilactici* reduced inflammation in colon tissue, through regulation of cytokine and chemokine release and partial modulation of microbial composition.

Collectively, our findings demonstrate that lactic acid bacteria play a beneficial role in mitigating metabolic or tissue damage-induced inflammation in the gut. Further investigation into the precise mechanisms that dictate their anti-inflammatory effects will deepen our understanding of their potential therapeutic applications in inflammatory pathologies.

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Breeding for resistance against Tobacco mosaic virus by silencing cytoplasmic *phosphoglycerate kinase* (*PGK*) genes in tobacco plants

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Plant viral diseases are among the most serious biotic stresses that lead to severe yield losses and economic damage, affecting global agriculture. Conventional breeding for virus resistance in plants is limited by the lack of natural resistance genes and the narrow genetic diversity within cultivated species. Consequently, the study of host susceptibility genes (S-genes) has gained increasing attention as a promising strategy to engineer durable resistance. Cytoplasmic phosphoglycerate kinase (PGK), possess ATP-generating activity and has been proposed as a putative susceptibility factor that viruses may exploit to support their cellular replication and/or movement. There are four cytoplasmic-*PGK* genes. The present study aims to elucidate the role of *PGK* genes in the interaction between *Nicotiana tabacum* and Tobacco mosaic virus (TMV). To achieve this, two complementary approaches were employed: Virus-Induced Gene Silencing (VIGS) and CRISPR/Cas9 genome editing. The *PGK* genes were silenced using a TRV-based VIGS system, and silenced plants were challenged with TMV. Quantification of viral accumulation was performed through RT-qPCR, and differences in symptom severity were recorded. The *PGK*-silenced plants had reduced TMV titers, confirming *PGKs* as potential host susceptibility genes. In addition, specific guide RNAs (gRNAs) targeting conserved regions of the *PGK* genes were tested *in vitro* for cleavage efficiency. The selected gRNAs were assembled with Cas9 into two different plasmid backbones: one lacking a replicon and one containing a Tomato leaf curl virus (ToLCV)-based replicon to enhance expression. Transient delivery of the assembled constructs in tobacco leaves via agroinfiltration enabled the evaluation of editing efficiency at the *PGK* genes via NGS sequencing. Future experiments are under way to perform transient assays for *PGK* editing and subsequent challenging with TMV. This study contributed to a better understanding of tobacco–TMV interactions and may provide a molecular basis for the development of TMV-resistant tobacco through targeted genome editing.

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P10 - 241

A rapid Agrobacterium-mediated transient expression approach for non-model crop species

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Agrobacterium tumefaciens is a Gram-negative bacterium that has served as a biotechnological tool of great importance due to its ability to transfer DNA into plant cells. Infiltration of plant tissues with Agrobacteria harbouring genes of interest (GOIs) has enabled researchers to generate transgenic organisms or to transiently express genes in specific organs for short periods. Advancements in plant virology have led to the development of deconstructed vectors encompassing genetic elements from various plant viruses, greatly enhancing the expression efficiency of transient GOIs in either model or non-model plants that are often recalcitrant to transformation. In this study, we investigated the possibility of transiently introducing GOIs into agronomically important non-model plants using plasmids encompassing viral genetic elements and the Green Fluorescent Protein (GFP) as a marker. Specifically, we used three different vectors (pTRBO-GFP, pHREAC-GFP and pEFF-GFP) in parallel with the appropriate negative controls (i.e., empty vectors) to agroinfiltrate leaves of tomato (*Solanum lycopersicum*; Solanaceae), eggplant (*Solanum melongena*; Solanaceae), lettuce (*Lactuca sativa*; Asteraceae) and spinach (*Spinacia oleracea*; Amaranthaceae). GFP expression was monitored macroscopically with a UV-Lamp and corroborated by confocal laser scanning microscopy showing correct intracellular expression of GFP. Our analyses revealed GFP expression in leaves of all tested plants, albeit with varying efficiencies. Under the experimental conditions tested, high levels of transient GFP expression were achieved when tomato and spinach leaves were agroinfiltrated with pTRBO-GFP, while high levels of GFP expression were observed in eggplant and lettuce leaves agroinfiltrated with pEFF-GFP. This study provides a valuable biotechnological approach to researchers working with these plant species enabling rapid *in planta* expression of GOIs, facilitating functional characterization of candidate genes - without the need for heterologous systems - and potentially allowing the manipulation of agronomically important traits.



P11 - 247

Metabolic characterization of the CO_2-catabolizing anaerobe *Moorella thermoacetica*'s growth in bioprocessing environments

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Non-photosynthetic CO_2 fixation by extremophiles has gained significant interest in biotechnological research over the past decade, as an alternative to biomass-based microbial processes. The relevant microbial systems catabolize CO_2 through the acetyl-CoA reduction pathway, aka Wood-Ljungdahl pathway, under strictly anaerobic conditions; the thermophile obligatory anaerobe *Moorella thermoacetica* has been used as the model system of these acetogens due to its small fully-sequenced genome. However, its physiology has not been extensively studied with high-throughput biomolecular analyses, particularly in the context of industrially-relevant environments, interesting for the optimization of biotechnological processes and settings. Omic data, especially metabolic profiles, analyzed in the context of the biomolecular networks, are essential to further our understanding of bacterial molecular physiology and regulation. For *M. thermoacetica*, the metabolic and protein networks are available, the latter recently reconstructed by our group, however, not extensive metabolic profiling data, especially under biomanufacturing-relevant conditions currently exist.

Our group is currently investigating an integrated CO_2 capture and bio-assimilation process, which can be useful to lower the carbon fingerprint of many gas-polluting industries, in the context of the HFRI-funded project, CO2BION, thus we are interested in profiling the physiology of *M. thermoacetica* in mixotrophic cultures with or without CO_2, along with its response to toxic heavy metal traces present in industrial emissions. In this study, we will show the establishment of a systematic protocol for the growth and metabolic profiling of this bacterium along with metabolomic data characterizing its growth in various conditions.

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Synergy between proneural and ectodermal transcription factors and the role of Notch in the early neurogenesis of *Drosophila*

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Early neurogenesis in *Drosophila melanogaster* is governed by the antagonistic interplay between the Notch signaling pathway (and its downstream E(spl)/Hes targets) and the proneural transcription factors (TFs) of the Achaete-Scute Complex (AS-C). The latter, display high evolutionary conservation with the mammalian ASCL, which, apart from promoting neurogenesis, are also implicated in human oncogenesis. Neurogenesis in the *Drosophila* embryo begins with the generation of the neural progenitors, called neuroblasts (NBs), which delaminate from the naïve embryonic neuroectoderm. The *AS-C* genes promote the neural fate determination, while Notch counteracts it by repressing the proneural activity and promoting the alternative epidermal fate via the process of lateral inhibition, ensuring that only a subset of cells becomes NB. It has been shown that proneurals are dispensable for the NB delamination process, their main role being the timely initiation of transcription, to promote stemness. In the absence of *AS-C*, NBs do acquire their stemness markers, albeit with a delay. This suggests that other, as yet unknown factors can mediate neurogenesis independently of the *AS-C*. To assess this, we used *AS-C*$^{-}$ mutants and crossed them with mutant genes of candidate TFs expressed in the neuroectoderm, namely *snail*, *SoxNeuro*, *daughterless* and *Krüppel*. Immunohistochemistry indicated worse defects in the phenotype of all double mutants compared to *AS-C*$^{-}$ single mutant, confirming that the studied TFs mediate neurogenesis in parallel to the proneurals. Furthermore, we overexpressed constitutively active Notch in the ventral neuroectoderm, which has the same effect as loss of the *AS-C*. We used RNA-seq and ATAC-seq analysis to examine the effects at the transcriptome and chromatin levels. Our results indicated that Notch causes mostly transcriptional repression without driving major chromatin remodeling. Integrating these findings, we suggest a revised gene regulatory network for early *Drosophila* neurogenesis, highlighting the interplay of Notch, AS-C and ectodermal TFs in neural fate determination.



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Evolutionary and Functional Insights into Primary Cilium-Mediated Regulation of Cortical Development

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Cortical development is one of the most intriguing and complex biological processes. Neural progenitor cells called radial-glial cells proliferate near the apical surface of the cortex (ventricular zone) and differentiate directly into neurons or give rise to intermediate progenitors (IP) or —only in primates— basal radial-glial cells, which in turn will eventually differentiate into neurons. Neurons migrate basally in an organized inside-out manner to populate the various layers of the cortex. In recent years, the primary cilium has emerged as major player in our understanding of cortical development. It is a non-motile thread-like cellular structure crucial for detecting and integrating signals from the extracellular environment consisting of a cell membrane protrusion surrounding a complex cylindrical structure of microtubules. It has attracted much attention because ciliary defects lead to a number of clinical conditions, characterised by malformations of cortical development (MCDs), such as microcephaly, periventricular heterotopia, and polymicrogyria. Several ciliary genes have been associated with MCDs, from which in our study we focus on *AHI1* and *OFD1*. They code proteins that localise in different compartments of the centrosome-cilia axis, thus making for an interesting target. Species-specific differences in expression patterns from single cell RNA sequencing analysis that we have performed, suggest that these genes may be important in the evolution of brain development among primates. Aiming to delve into the functional role of primary cilium in brain development, we manipulated the expression of these genes in mice and human brain organoids. We found that dysregulation of these genes induces changes in the size and distribution of the population of neural progenitors and neurons. Our results indicate that the cell cycle in progenitor cells is also affected. Collectively, our findings provide a base for new mechanistic insights on the multifaceted role of primary cilium in cortical development and disease manifestation.



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Effect of mechanical loading and/or treatment with natural/synthetic compounds on human cells with osteoblastic phenotype

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Bone remodeling functions as an ongoing process throughout life to preserve skeletal strength. Bone remodeling processes are particularly influenced by mechanical loading. Bone tissue cells possess the ability to detect mechanical stimuli including tension and compression forces. Therefore, bone cells initiate signaling pathways which interfere with gene expression and cell differentiation processes.

As recent scientific interest focuses on the effect of natural and synthetic antioxidant molecules on osteoblast physiology, the aim of the present study was to investigate a) how MG-63 osteoblast-like cells respond to mechanical stimulation and b) how the natural polyphenol hydroxytyrosol (HT) and the HT synthetic analog (FR503) impact this cellular response.

MG-63 cells were plated in special collagen-coated plates, were treated with HT and FR and were then exposed to cyclic tensile strain for 12 hours using a cell-stretching apparatus (Flexcell Tension System). Fluorescence-activated cell sorting (FACS) was used to analyze cell cycle distribution and apoptosis in both treated and control MG-63 cells. Finally, the alteration of the expression of five selected bone-related genes were evaluated by Real-Time PCR.

Our results revealed the protective role of mechanical loading in the MG-63 osteoblast-like cells distribution and in reducing apoptosis in all tested conditions. Additionally, enhanced gene expression of osteocalcin (OSC), bone-specific alkaline phosphatase (ALP) and bone morphogenetic protein 7(BMP7) were observed after combining mechanical loading and HT and FR503 treatment conditions.

Our findings suggest that more profound studies of these loading-mediated cellular responses might help the development of new approaches to enhance bone re-generation and tackle bone-related diseases.



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The role of BCL6 in the transcriptional control of invariant Natural Killer T (iNKT) cell development

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Invariant Natural Killer T (iNKT) cells are a distinct subset of T cells that exhibit features of both branches of immunity, playing a unique role in immune regulation. Their development diverges from conventional T cells in the thymus at the stage of DP progenitor cells. Following positive selection, iNKT cells undergo a stepwise differentiation pathway that gives rise to distinct mature subsets with diverse effector potentials; iNKT1, iNKT2 and iNKT17. BCL6 is a transcription factor transiently expressed in progenitor iNKT cells that plays a crucial role in establishing a chromatin landscape suitable for the expression of key genes.

Our goal is to examine how BCL6 may contribute to gene regulatory networks that control subsequent iNKT sublineage diversification and orchestrate gene regulatory networks during iNKT cell development. To clarify the role of BCL6 in the molecular events that coordinate the development of iNKT cells we have conducted integrative single-cell genome-wide approaches (scRNA-seq and scATAC-seq) in mice that lack the transcription factor BCL6 via the CD4Cre system ($CD4^{Cre}Bcl6^{F/F}$), mice that ectopically express BCL6 under the *Lck* promoter ($Bcl6^{Tg}$) and *WT* mice. In addition, we have conducted Flow cytometry analysis to analyse the iNKT developmental stages in thymus from $Bcl6^{Tg}$ and *WT* mice.

Results: We observed a skewing of iNKT cell differentiation toward the iNKT2 lineage and a corresponding reduction in iNKT1 cell commitment in mice that ectopically express BCL6. Conversely, mice that lack Bcl6 exhibit an enhanced iNKT1 transcriptional profile, even within iNKT2 populations, suggesting a role for Bcl6 in suppressing premature iNKT1 maturation.

Our work highlights BCL6 as a key transcriptional regulator that orchestrates iNKT cell differentiation. These insights could inform future therapeutic approaches targeting BCL6 in the context of autoimmune disorders, infections, and cancer.



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LEFKOTHEA regulates the mode of alternative splicing events during light response

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Light is the source of energy for photosynthetic organisms such as plants. Due to the great significance of light in the regulation of plant development, plants possess a sophisticated signaling network that controls light response including a massive gene expression reprogramming as well as post-transcriptional mechanisms such as alternative splicing. LEFKOTHEA is a nucleus-encoded splicing factor found in both the nucleus and chloroplast and its nuclear compartmentalization ensures the early embryonic development in *Arabidopsis*. *LEFKOTHEA* gene mutations (*lefko1*, an EMS mutant allele exhibiting aberrant nuclear partitioning and an albino cotyledon phenotype, and *lefko2*, a severe allele that results in embryo developmental arrest) have defects in splicing of nuclear and chloroplast genes. Phenotypic analysis of *lefko1* at the early developmental stages showed defects in the primary root elongation of light-grown seedlings, as well as longer hypocotyls and delayed photosynthetic pigments accumulation, suggesting a delayed response to light. Further, confocal microscopy demonstrated that light is a crucial regulator of LEFKOTHEA nuclear localization, which is also influenced by cell type and differentiation state. Time-series transcriptomic analysis upon light encounter revealed an aberrant response to light in *lefko1*, characterized by down-regulation of genes involved in photosynthetic apparatus and signal transduction pathways. LEFKOTHEA significantly affects the number and the mode of alternative splicing events during light response. A majority of such events occur on spliceosomal components, light-related transcription factors and genes involved in signal transduction. The fact that less than 5% of differentially expressed genes upon light encounter exhibit splicing defects indicates that LEFKOTHEA modulates alternative splicing events on a subset of differentially expressed genes, crucial for proper light response and development. Altogether, our results show that the nuclear compartmentalization of LEFKOTHEA is essential for the establishment of both the transcriptional and post-transcriptional landscapes that facilitate the response to light at early developmental stages.

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Non-Invasive Multi-Omics Approaches for Comprehensive Non-Invasive Assessment of Human Preimplantation Embryos. Challenges, Limitations and Planning of a Prospective Collaboration Between Hygeia IVF Embryogenesis and BRFAA

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Background/Aim: Success of in vitro fertilization (IVF) depends on accurate embryo selection prior to transfer. Current methods, including morphology-based scoring or invasive genetic testing, are limited by subjectivity or potential harm to embryo integrity. Development of reliable, non-invasive technologies is a challenge in assisted reproductive technology. Omics technologies enable detection of molecular biomarkers released by the embryo in the culture medium during in vitro culture. This report explores the challenges and limitations that are expected to affect multi-omics assessment of spent embryo culture media and addresses planning issues in a prospective collaboration between two major Greek institutions: Hygeia IVF Embryogenesis and the Biomedical Research Foundation of the Academy of Athens (BRFAA).

Materials and Methods: Following oocyte retrieval, mature oocytes will be fertilized by intracytoplasmic sperm injection. Oocytes and resulting embryos will be cultured individually in wells with 20 μl culture medium in a time-lapse incubator. Embryo-conditioned spent media will be collected aseptically between Day 3 and Day 5 of embryo culture at Hygeia IVF Embryogenesis. Culture media samples free of contamination will be aspirated into sterile cryovials, snap-frozen in liquid nitrogen, and transported under cryogenic conditions to BRFAA's Multi-Omics Laboratory. Samples will be processed upon admission. Proteomic analysis will be carried out using a modified filter-aided sample preparation protocol, tryptic digestion, and analysis by nano-liquid chromatography coupled with high-resolution Orbitrap mass spectrometry. Data will be processed with Proteome Discoverer and R-based bioinformatics tools to quantify differentially expressed proteins. The study will be initiated following approval of the Scientific Boards of the two Institutions.

Anticipated Results: Several critical factors are expected to affect a successful protocol standardisation. (i) Minute sample volumes, low concentrations of embryo-derived molecules, and interference from culture medium, will be dealt with modifications in extraction buffers. (ii) Presence of albumin at high concentrations, suppressing low abundant molecules, will be overcome by use of relevant albumin removal kits. (iii) Absence of standardized analytical protocols affecting reproducibility and sample integrity will be addressed by careful planning of both the collection and transfer processes, and streamlining of the analytical protocol.

Conclusion: Non-invasive multi-omics approaches present the potential of a precise and ethical framework for non-invasive embryo selection. The performance of the technology may be further augmented by combining multi-omics analysis with AI-driven interpretation. Hygeia IVF Embryogenesis and BRFAA will successfully collaborate towards this path.



P18 - 12

Unraveling interactions between DNA Damage Response network and Immune System in Multiple Myeloma

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Multiple myeloma (MM) is a hematologic malignancy that, despite therapeutic advances, remains incurable due to treatment resistance and inevitable relapse. Both the DNA Damage Response (DDR) network and the immune system are essential for cellular homeostasis, and emerging evidence suggests functional crosstalk between these mechanisms. Since recent data have shown that DNA-damaging agents can enhance tumor immunogenicity and stimulate antitumor immunity, herein, we investigated changes in key molecular mechanisms underlying the activation of the immune system following the detection of DNA damage.

At baseline and following treatment with genotoxic drugs (Bortezomib, Carfilzomib, Melphalan and Oxaliplatin) DNA damage, interstrand crosslink repair (ICL/R), γH2AX accumulation, oxidative stress and apurinic/apyrimidinic (AP) sites were quantified in several MM cell lines and a healthy B lymphoblastoid cell line. Flow cytometry was employed to determine whether these drugs induced immunogenic cell death (ICD), altered MHC class I expression, or modulated the immune checkpoint protein PD-L1 on MM cells. Finally, the release of cytoplasmic double-stranded DNA (dsDNA) and the induction of the cGAS–STING pathway were analyzed.

Genotoxic treatments increased DNA damage, AP-sites, and oxidative stress across all MM cell lines. Chemotherapeutic agents triggered ICD, evidenced by higher ecto-calreticulin and HSP90 exposure, and enhanced antigen presentation via MHC class I upregulation. Increased cytosolic dsDNA levels across all MM cell lines compared to the control cell line were observed. In several MM cell lines, PD-L1 expression was elevated, suggesting activation of adaptive immune resistance. Conversely, treatment suppressed cGAS–STING pathway activity in MM cells. Together, the DDR network is implicated in the immunostimulatory effects of certain chemotherapy drugs, as DNA damage activates several immune pathways. This interaction is crucial as it provides a dual mechanism for controlling cancer by not only triggering cancer cell death but also stimulating the immune system to destroy tumor cells.

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DNA Damage Response and redox status reveal determinants of treatment sensitivity in multiple myeloma cells

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Resistance to DNA-damaging agents remains a major challenge in multiple myeloma (MM) therapy. The DNA Damage Response (DDR) network is a complex system responsible for maintaining genomic stability, and its dysregulation is strongly linked to cancer development, progression, and treatment resistance. Oxidative stress, defined as an imbalance between the production of reactive oxygen species and the cells' ability to detoxify them, can cause damage to cellular components, leading to mutations and promoting abnormal cell growth. DDR and redox status play crucial roles in determining cell fate following genotoxic stress, however, their interaction in MM remains poorly understood. Here, we investigated how DDR-related signals and redox status influence the sensitivity of MM cell lines to genotoxic insults. At baseline and after exposure to ultraviolet C irradiation or melphalan, we assessed DNA damage accumulation, DNA repair efficiency [Nucleotide excision repair (NER), interstrand crosslink repair (ICL/R) and double-strand breaks repair (DSB/R)], chromatin condensation, redox status, and apoptotic rates in a panel of eleven MM cell lines and one healthy B-lymphoblastoid control. In addition, exploratory statistical analyses, including correlation and hierarchical clustering, were applied to capture potential interactions among these parameters. The analysis revealed that MM cell lines with higher apoptosis rates are characterized by higher levels of baseline DNA damage, reduced NER and ICL/R capacities, more condensed chromatin structure, reduced redox status, and augmented apurinic/apyrimidinic lesions. Correlation analysis revealed strong trends among the observed markers, and combined with hierarchical clustering, we were able to group our samples according to their redox and DDR status. Collectively, our findings indicate that DDR efficiency and redox status act in concert to shape apoptosis susceptibility and treatment response in MM, underscoring their potential as functional biomarkers of therapeutic sensitivity.

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The regulation of McIdas protein levels is critical for normal cell cycle progression and the maintenance of genomic integrity

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Chromosome duplication and centrosome amplification must be tightly regulated in order to safeguard genomic stability[1]. Aberrations in genome replication or centriole biogenesis perturb normal cell cycle progression, leading to the emergence of genomic instability that is linked to tumorigenesis[1,2]. McIdas, a nuclear protein that belongs to the Geminin superfamily, has been previously characterized for its significant roles in centrosome duplication and cell cycle control[2,3]. Its protein levels are reduced during anaphase and remain low until the end of mitosis whereas they increase upon entry in G1 phase, reminiscent of the pattern followed by APC/C substrates[1]. Previous work of our lab showed that McIdas ectopic expression is associated with defective mitotic phenotypes and genome content anomalies[3]. In this regard, this study focused on elucidating the regulation of McIdas during the cell cycle and the implications of its perturbance for cellular integrity. The depletion of CDC20, a known activator of the anaphase promoting complex, in HeLa cells led to the stabilization of McIdas during mitosis. This observation indicates that APC/C is involved in the regulation of its protein levels. Additionally, the expression of non-degradable mutant forms of McIdas resulted in genome instability phenotypes, as demonstrated by the increased occurrence of micronuclei, chromatin bridges and 53BP1-containing nuclear bodies, along with the formation of multipolar mitotic spindles and multinucleated cells. Lastly, clonogenic assays conducted on cells stably expressing the non-degradable forms of McIdas revealed a decreased tendency for colony formation, highlighting the impact of McIdas deregulation on cell growth. Based on these findings, further research will focus on unraveling the mechanisms that are implicated in the multiple roles of McIdas during the cell cycle, which will allow a better understanding of the contribution of the Geminin family to the maintenance of genomic integrity.

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Utilizing predictable staggered CRISPR/Cas9 cuts for precise genome editing

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CRISPR–Cas9 has transformed genome editing in both fundamental and applied biomedical research, offering a programmable and precise method for correcting genetic disorders. Despite advances in improving DNA-targeting specificity through redesigned guide RNAs (gRNAs) and high-fidelity Cas9 variants, achieving precise, template-free editing in eukaryotic cells remains a challenge for therapeutic applications mostly due to low editing efficiency, genomic instability and low predictability of the repair outcome. We have recently established BreakTag (Longo et al., *Nat. Biotech*, 2025), a novel, next-generation based methodology capable of mapping the frequency, location and end-structure of Cas9-induced DSBs across the genome. Using BreakTag to profile Cas9-induced DNA cuts across multiple genomic sites, we found that the protospacer sequence dictates Cas9 scission profile and repair outcome, with staggered cuts being repaired predominantly as templated, single nucleotide insertions. We have also identified a Cas9 engineered variant with skewed cleavage properties toward staggered cuts, which can be used for mediating precise and templated insertions at targeted sites with specific protospacer sequence profile. Since staggered cuts can improve predictability of +1 nucleotide insertions, changing the open reading frame resulting in higher efficiency of knockouts (KO), we have used this approach to identify novel target sites for efficient KO of genes to improve cancer activity and efficiency of CAR-T cells. Moreover, we are currently further optimizing the repair of Cas9-induced staggered cuts towards +1 insertions by manipulating competitive DNA repair pathways. To achieve this, we have established cell-based fluorescent reporter systems which we use in combination with genome-wide CRISPR/Cas screens to identify molecular factors that specifically suppress +1 nucleotide insertions. Ultimately, our goal is by controlling Cas9 scission profile towards staggered cuts to achieve more predictable, more precise and safer gene editing, offering potential treatments for genetic diseases and improving targeted cancer therapies.

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miRNA profiling and genotoxicity screening of extracellular vesicles derived from human infrapatellar fat pad mesenchymal stem/stromal cells

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Mesenchymal stem/stromal cells (MSC) have garnered attention for their potential in regenerative medicine due to their immunomodulatory and anabolic properties[1]. Extracellular vesicles (EVs) serve as vehicles for cellular export products, and can modulate the function of other cells at proximal or distal sites[2]. The immunomodulatory, angiogenic, and anabolic capabilities of MSC-EVs are well documented through cargo analysis and preclinical studies, supporting their use instead of MSC in clinical trials[3-9]. Recent studies have shown that EVs may enhance tissue regeneration and could therefore function as a cell-free alternative in injury healing. However, their cargo molecules have not been fully characterized, whereas the genetic toxicity of MSC-EVs remains unknown. In this study, human MSC-EVs originating from infrapatellar fat pad tissue (IFP) were examined for their miRNA content, as well their genotoxicity by performing two different *in vitro* cell-based assays using human osteosarcoma(U2OS) cells. The first was an immunofluorescence assay based the DNA damage marker γH2AX. The second, called micronucleus assay, involved the analysis of small nuclei, representing broken DNA fragments, that appear next to the main nucleus of the cells. MSC-EVs from IFP tissue showed distinct miRNA immunomodulatory profiles. Reactome analysis of miRNAs highly present in EVs showed their involvement in the regulation of six gene groups in target cells, particularly the immune system. EVs internalization was confirmed by staining exosomes with a lipophilic dye before exposing them to the target cells. MSC-EVs were shown not to be genotoxic and therefore did not activate a DNA damage response pathway in U2OS cells. Taken together, our data show that MSC-EVs show robust miRNA immunomodulatory profiles and do not induce a DNA damage response, suggesting that their cargo does not include factors that could activate this pathway. Based on MSC-EVs strong paracrine immunomodulatory/trophic 'medicinal' activities, we propose a viable cell-free alternative to MSC-based therapeutics.

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Unresolved telomere recombination in cancer leads to ultrabright telomere-driven cellular senescence

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Activation of telomere maintenance mechanisms is essential for limitless proliferation of cancer cells. A subset of aggressive malignancies employs the recombination-driven Alternative Lengthening of Telomeres ALT-pathway. Ultrabright Telomeres (UT) and single-stranded telomere clustering, define histopathological and cytological diagnostic hallmarks of ALT. However, up to date the mechanistic basis of these two phenotypes and their biological significance remain unresolved. Herein we reveal that the ALT-associated UT recruit the recombinases RAD51, RAD52, the DNA damage response molecules γ-H2AX and 53BP1, the single-stranded DNA binding proteins RPA1 and ATRIP and the structure specific nucleases SLX4, MUS81 and BLM. Furthermore, we show that the frequencies of ALT nuclei with UT become markedly elevated upon oncogene-induced replication stress and loss or suppression of key ALT regulators like RAD52, ATR and MRE11. These data establish UT as persistent, under-processed, dysfunctional recombination intermediates, representing telomere entanglements rather than productive ALT. Interestingly, UT-bearing cancer cells exhibit a multitude of senescent phenotypes and display highly increased sensitivity to senolytic drugs. Our results reveal for the first time, a previously unknown mechanism of neoplasia associated cellular senescence arising from entangled telomeres of different chromosomes that can be detected in cancer specimens as hyperfluorescent telomeric signals. These novel findings provide mechanistic clarity to ALT diagnostics suggesting in parallel that senolytics may become efficient countermeasures against dormant cancer cells that have the capacity to escape traditional oncotherapeutics.



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The anatomical site, the developmental stage and the differentiation state direct the vulnerability of human mesenchymal cells to UVB radiation

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The skin, due to its outermost anatomical location, is continuously exposed to solar ultraviolet (UV) radiation. The more energetic UVB wavelength (280–315nm) is mainly absorbed by the epidermis; however, an ~5–10% of its photons reach and affect the upper part of the dermis. On the other hand, UVB radiation is considered as the main effector of premature skin aging (the so-called photoageing), characterized by skin fibroblast cells' death and premature senescence. We have previously shown that physiologically relevant UVB radiation doses are genotoxic and provoke apoptosis in human skin fibroblasts, while the cells possess an arsenal of synergistically acting protective signaling pathways, with JNKs/ATM-p53 activation and interplay being indispensable for the perpetuation of cellular defense towards UVB radiation and the retainment of cell viability, while EGFR/Akt and Nrf2 being auxiliary anti-apoptotic machineries [Cell Death & Dis. (2022) 13: 647]. Aim of this work was to compare the vulnerability of human fibroblasts derived from tissues being exposed to different levels of UVB radiation. Our data show that fibroblasts from the face are more resistant compared to those derived from the arm or the lung (LD_{50}: 181±13 vs 157±12 vs 103±15 mJ/cm^2, respectively). Interestingly, skin and lung fibroblasts from human fetuses are much more vulnerable to UVB radiation (LD_{50}: 22±3 and 18±3 mJ/cm^2, respectively). Even more intriguing, human adult adipose-derived mesenchymal stem cells have similar LD_{50} values with fetal fibroblasts (approx. 22 mJ/cm^2). These data indicate that the anatomical position, the developmental origin and the level of differentiation direct the vulnerability of human fibroblasts to UVB radiation. The mechanisms underlying the above-mentioned differences – with an emphasis in the anti-oxidant capacity and the response to genotoxic stresses – are currently under investigation.



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Targeting Replication Licensing Machinery for the Development of Cancer-Specific Therapeutic Approaches

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Replication licensing is a strictly regulated mechanism that ensures DNA is replicated only once-per-cell-cycle, thereby maintaining genome integrity[1]. CDT1 is an essential licensing factor that facilitates timely recruitment of MCM helicases onto chromatin, and is tightly regulated through selective mechanisms, preventing illegitimate origin firing. One such regulatory mechanism involves Geminin, a protein that inhibits CDT1 by direct binding[2]. Previous results have revealed that cancer cells, where CDT1 is frequently upregulated, rely on Geminin to prevent re-replication, as its depletion leads to severe replication stress[3]. In this context, we investigated the use of AF615, a small-molecule inhibitor, developed by our group, targeting Geminin/CDT1 complex[4]. Incubation of U2OS cancer cells with AF615 resulted in a significant, dose-dependent accumulation of DNA double-strand breaks (DSBs), an effect that was not observed in normal hTERT-RPE1 cells. Additionally, incidents of re-replication and reduction of cell viability were observed, selectively in cancer cells. The minimal effects observed after incubation of AF615 in normal cells, are significantly increased upon CDT1 overexpression, which more closely resembles CDT1 levels in cancer cells. Simultaneous use of the ATR-inhibitor VE-821, or the PARP-inhibitor Olaparib further sensitizes cancer cells, in contrast to normal cells, leading to a severe reduction in cell viability and accumulation of DNA damage. To further increase the potency of AF615, our group developed analogue compounds, that carry the same core as the lead compound and bear differences in their side chains. Comparative analysis between AF615 and the analogues will demonstrate the efficacy of analogues in inducing DNA damage and genomic instability. Our results indicate that small molecule inhibitors targeting the Geminin/CDT1 complex could be employed as a method to selectively sensitize cancer cells, either as monotherapy, or in combination with other known anti-cancer agents, leading towards a novel path in cancer therapies.

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P26 - 21

MCU and mPTP mediate cytosolic calcium overload and neuronal dysfunction in *C. elegans* models of autosomal dominant optic atrophy

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Autosomal Dominant Optic Atrophy (ADOA) is an inherited neurodegenerative disorder characterized by progressive vision loss due to degeneration of the optic nerve. The disease is caused by mutations in the *OPA1* gene, which regulates the dynamics of the inner mitochondrial membrane. Recent studies have revealed that the symptoms of ADOA are driven by elevated cytosolic calcium levels. However, the mechanism underlying the increase in cytosolic calcium remains unknown. To investigate this mechanism, we used a *Caenorhabditis elegans* strain carrying a mutation in the *eat-3* gene, the ortholog of the human *OPA1* gene. The *eat-3(ad426)* mutation resulted in significantly elevated cytosolic calcium levels, successfully recapitulating this specific phenotype of ADOA. The mitochondrial calcium uniporter (MCU) was found to be essential for the observed cytosolic calcium elevation in the *eat-3* mutant. Furthermore, subunits of the mitochondrial permeability transition pore (mPTP), such as the adenine nucleotide translocator (ANT) and cyclophilin D, but not the ATP synthase, were also found to be required for the increase in cytosolic calcium. The mitochondrial calcium transporters NCLX and LETM1 did not appear to contribute. We also examined a nematode strain expressing the human mutant protein OPA1^{K301A} in GABAergic neurons. Expression of OPA1^{K301A} disrupted the defecation cycle, indicating GABAergic neuron dysfunction. Inhibition of MCU using RU360 reduced neuronal dysfunction, while inhibition of mPTP by cyclosporin A fully restored neuronal function. These findings highlight the MCU and the mPTP complex as potential therapeutic targets and outline a mechanistic model for the pathophysiology of ADOA. We propose that mitochondria take up large amounts of calcium via MCU and subsequently release it into the cytosol upon mPTP activation, leading to cytosolic calcium overload and neuronal impairment.



P27 - 24

Development of a real-time PCR method for the quantitative determination of sjTREC episomal DNA in the thymus

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Signal joint T cell receptor excision circles (sjTRECs) are circular episomal DNA molecules, generated during a DNA recombination process that T cells employ to produce antigen-specific α/β T cell receptors. Since 70% of newly formed mature TCR αβ+ lymphocytes carry sjTRECs, which lack the ability to replicate during T cell proliferation, these DNA episomes function as markers of recently produced T cells and thymic output. Currently, probe-based PCR methods are widely used for quantifying sjTRECs both in the thymus and in peripheral blood. In the present study, we developed a PCR assay based on SYBR Green chemistry, an approach that offers a cost-effective alternative without compromising sensitivity and specificity. The number of sjTREC amplicons were determined in the thymus of C57Bl6/NCrl mice and normalized by the number of copies of the TCRa gene. To test this assay, we performed two separate experiments, in which mice were subjected to factors known to induce thymic involution, i.e. aging and corticosteroid administration. In the first experiment, sjTRECs were quantified in groups of young (6-7 weeks) and old (95-96 weeks) mice. The results revealed that sjTREC copies were significantly lower in the aged mice ($p<0.0001$), which paralleled thymus weight index reduction in the same group. In the second experiment, the corticosteroid dexamethasone led to a severe drop in sjTREC levels, observed at day 1 after administration ($p=0.002$), that correlated with the expected thymus weight index decrease at the same time point ($p=0.0015$). Although no difference between groups was identified upon thymic regeneration at 15 days post-treatment, sjTRECs never recovered to their initial levels. In conclusion, a quantitative PCR assay was developed, which determines sjTREC levels that reliably reflect thymic output in mice. Such quantification may provide a significant insight into thymic function and the impact of various immune-modulating factors on thymic output.



P28 - 26

Cracking the Apoptosis Code: Deciphering how ORF3a mutants orchestrate the RIPK1 signaling dynamics

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ORF3a is a multifunctional SARS-CoV-2 accessory protein that is involved in viral pathogenesis by modulating cellular homeostatic mechanisms. Unlike spike protein, ORF3a exhibits only point mutations in its structure, which remain conserved within each variant, thus making it a more approachable target for mechanistic studies. This study addresses how ORF3a (Wuhan) protein and its natural mutants (Beta, Gamma, Delta, and Omicron) regulate RIPK-1-dependent apoptotic signaling in human epithelial cells. In A549, lung, and Caco-2, intestinal, epithelial cells transfected with the respective ORF3a mRNAs, it was established that all ORF3a proteins activate the RIPK1 kinase, yet to varying extents, as indicated by elevated phosphorylation levels at the Ser166 residue. Live/Dead staining and Western blot analyses demonstrated that all mutations induce apoptotic cell death, as indicated by the cleavage of caspase-8, caspase-3, and PARP-1. Functional inhibition experiments were conducted in both cell lines to assess the dependence of ORF3a-induced apoptosis to the enhanced activity of RIPK1 kinase. Therefore, the transfected cells were either treated with 20 μM Necrostatin-1, a specific RIPK1 inhibitor, or with 50 μM z-VAD-fmk, a pan-caspase inhibitor. Furthermore, treatment of transfected, A549 and Caco-2 cells with z-VAD-fmk abolished caspases-8, -3, and PARP-1 cleavage, whereas Necrostatin-1 treatment inhibited RIPK1 phosphorylation and partially suppressed caspase cleavage. Indeed, these findings indicate that the ORF3a-induced apoptosis is initiated upstream by RIPK1 and subsequently executed by caspases. Taken together, by linking alterations in ORF3a structure to the observed functional outcomes, ORF3a orchestrates host cell apoptosis through RIPK1 signaling, potentially leading to severe complications during SARS-CoV-2 infections such as tissue damage and cytokine storm.

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Uncovering the pathophysiology of cerebellar ataxia with neuropathy and vestibular areflexia syndrome (CANVAS) using patient-derived brain organoids

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Neurological disorders are a leading cause of disability worldwide. Ataxias, in particular, impair muscle coordination and voluntary movements, including walking, speaking, swallowing, and control of eye movements. Among them, cerebellar ataxia with neuropathy and vestibular areflexia syndrome (CANVAS) is a late-onset hereditary condition characterized by cerebellar, bilateral vestibular, and somatosensory deficits. Recent studies have identified biallelic repeat expansions in the replication factor C subunit 1 (RFC1) gene in CANVAS patients. RFC1 is a DNA-polymerase accessory protein that is crucial for DNA replication, repair of lesions occurring during replication and promoting the cell cycle. In order to evaluate the expression patterns of RFC1 during brain development we performed analyses on published single-cell and bulk RNA sequencing data in cells isolated from human brain organoids in different developmental stages. Our analyses showed that RFC1 is strongly expressed during early brain development, and its expression coincides with the presence of progenitor cells. So, given RFC1's role in DNA replication and repair, we hypothesize that its mutation may disrupt early molecular and cellular processes in the nervous system, prior to disease manifestation. To this end, we generated induced pluripotent stem cells (iPSCs) from healthy donors and patients with genetically confirmed CANVAS and then developed brain organoids (BOs) to model early cellular and molecular hallmarks of the disease. Patient-derived BOs exhibited reduced size compared to controls during early stages, suggesting developmental defects. Analyses of the BOs highlight the impact of RFC1's repeat expansions in the normal processes of neural progenitor proliferation and neuronal differentiation, while assessing their effects on DNA repair mechanisms. In addition, via a non-hypothesis-driven high-throughput approach we aim to further explore CANVAS etiopathology and uncover the earliest mechanisms underlying CANVAS, providing crucial insights into its pathophysiology and possibly identifying novel approaches to diagnosis and personalized treatment of patients.



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NLRP3- and sex-dependent inflammatory interplays contribute to adverse outcomes of cardiac repair

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During cardiac repair inflammatory pathways converge in major cardiac cell populations and determine the response to injury and relevant outcomes. We hypothesized that the NLR family pyrin domain containing 3 (NLRP3)-interleukin 1 (IL1) axis and inflammasome pathways are activated in a genetic mouse model of heart failure (HF) and may contribute to the cardiac output. Indeed, our previous molecular and histological analyses during the inflammatory phase of HF in desmin-deficient ($des^{-/-}$) mice have shown that NLRP3 inflammasome-related pathways are active in multiple cell types in the injured hearts. To address the above hypothesis, we crossed $des^{-/-}$ mice with *Nlrp3*-deficient mice and found that some of the echocardiography-based parameters indicating cardiac adverse remodeling and dysfunction, including the percentage of fractional shortening, as well as histological fibrosis were ameliorated in double $Nlrp3^{-/-}des^{-/-}$ knock outs as compared to $des^{-/-}$ counterparts. Interestingly, these improvements, under NLRP3 deficiency, were evident in male mice in contrast to what occurred in females, where parameters of cardiac function and myocardial fibrosis either remained unaffected or even marginally worsened. At the molecular level, differential expression patterns, between male and female hearts, of members of the NLRP3-IL1 axis are linked to cardiac inflammation, while components of alternative inflammasome systems, like *Aim2* and *Pyrin,* although elevated in the failing hearts did not significantly differ between genotypes or sexes. At the cellular level, cultured fibroblasts from the failing hearts expressed increased levels of *Nlrp3,* while those of male origin further stimulated collagen I expression when triggered *in vitro* to form active inflammasomes. In addition, sex-dependent differences in the response of cardiac fibroblasts to pro-fibrotic stimuli may also contribute to the cardioprotective effects of NLRP3 loss-of-function that are being preferentially exerted in males.



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Impact of Cold Atmospheric Plasma on lung cancer cells before and after TGFβ-induced EMT

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Cold Atmospheric Plasma (CAP) technology has recently emerged as an innovative approach in anticancer therapy. Studies in cellular and animal models have demonstrated that plasma induces selective death in cancer cells, attracting significant scientific interest as a potential therapeutic modality with reduced impact on normal tissues. CAP is an ionized gas where the ions are close to room temperature and its action is primarily attributed to the production of reactive oxygen and nitrogen species (ROS/RNS), which trigger oxidative stress and activate cell death mechanisms. Previous studies from our laboratory showed that CAP treatment in breast cancer cells leads to extracellular matrix (ECM) remodeling, alterations in cell morphology, and ultimately induction of apoptosis [1]. These findings prompted us to explore the potential application of CAP technology in the development of therapeutic strategies against metastatic lung cancer. To this aim, we investigated the effect of CAP on A549 lung cancer cells that acquire an enhanced metastatic potential following stimulation with transforming growth factor beta (TGFβ). CAP induced morphological changes and increased apoptosis rates in lung cancer cells of different phenotypes, i.e. before and after TGFβ-induced epithelial-to-mesenchymal transition (EMT). Moreover, significant changes were observed in the expression levels of key EMT markers, as a consequence of CAP-induced cytotoxicity. These changes were accompanied by substantial alterations in the tumor microenvironment, including the hyaluronan network (synthases, hyaluronidases, CD44 receptor) and metalloproteinases, with established roles in the metastatic process. Furthermore, comparison of cellular alterations before and after CAP treatment revealed a restriction of EMT program, indicating the potential contribution of plasma in preventing metastasis progression.

Overall, our results support that CAP is a promising therapeutic method for lung cancer and a potential strategy for restriction of metastasis.

[1] Aggelopoulos et al., Frontiers in Oncology, 2022 (doi: 10.3389/fonc.2021.826865)



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Functional Correlation of the Antigenic Properties of Oncogenic Proteins with Paraneoplastic Neurological Disorders

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Paraneoplastic neurological syndromes (PNS) are a group of rare autoimmune diseases that affect approximately 0.3% of cancer patients worldwide [1]. The cause of PNS is a misguided immune response targeting neurons and glia due to the recognition of the corresponding neuronal antigens in tumors where they are ectopically expressed. The antibodies produced are called onconeuronal autoantibodies and the most studied ones are the anti-Ri, anti-Yo, and anti-Hu, which respectively target the proteins NOVA-1 and NOVA-2, CDR2 and CDR2L and ELAVL4 [3].

The aim of our study is to determine the patterns and mechanism of ectopic neuronal antigens expression in cancer. Specifically, the differential expression of the target antigens between different cancer stages was examined using two cellular models of multistage carcinogenesis, HEK293T and SH-SY5Y cell lines. Additionally, the subcellular localization of these antigens was studied, along with potential changes in cell morphology during the course of carcinogenesis. Molecular analysis was performed using Western blotting and through Quantitative Real-Time Polymerase Chain Reaction (Q-RT-PCR). Imaging of the cells at each stage and cell type was conducted using immunofluorescence. The autoantibodies used to probe expression differences were derived from patient serum samples, which were characterized as positive for a specific type of antibody.

Our main finding was an increase in the expression of the Ri antigen during the progression of carcinogenesis in our cellular model and the observation of different staining patterns depending on the antibodies present in each serum. Similar results were obtained using immunofluorescence for the Yo antigen, while no significant differences were noted for the Hu antigen between the cell lines. More importantly we observed a different molecular pattern of expression of NOVA-1 and NOVA-2 in HEK293T and SH-SY5Y. These results may offer a way to distinguish which specific isoform of Ri antigen is the immunization agent in each and every patient tested.

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P33 - 79

Exploring integrins-matrix interactions in 2D and 3D cancer cell models

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This study aims to investigate how integrins and hyaluronic acid (HA)–derived fragments influence cancer cell behavior in 2D and 3D culture systems. Integrins are key mediators of cell–matrix interactions, governing adhesion, migration, and survival, and their dysregulation is strongly linked to tumor progression and therapeutic resistance. Given the importance of HA as a major ECM glycosaminoglycan involved in cell signaling and matrix organization, its bioactive derivatives represent promising modulators of tumor cell responses.

To address how dimensionality shapes these interactions, we compared 2D and 3D culture models, which revealed distinct expression patterns in integrins and other adhesion-related genes, highlighting the greater physiological relevance of 3D systems. Building on this, we applied fragments of 50 kDa HA and sulfated HA (sHA) both of which have demonstrated potent anti-cancer effects, to evaluate their impact on integrin-associated pathways and ECM remodeling across both culture platforms.

Overall, this work seeks to demonstrate the importance of the interin's–ECM–HA networks in cancer cell regulation, offering insight into the dimensional and biochemical factors that shape tumor progression and treatment response.



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Regulation of LRRK2 Kinase Activity and Dimerization by Rab GTPases in Parkinson's Disease

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Parkinson's disease (PD) is the second most common neurodegenerative disorder, primarily known for its progressive motor symptoms. Mutations in leucine-rich repeat kinase 2 (LRRK2), a kinase/GTPase, drive PD by causing excessive kinase activity towards multiple substrates, and cellular dysfunction. Rab GTPases coordinate most membrane trafficking processes in eukaryotic cells and are well-characterized LRRK2 substrates that become aberrantly phosphorylated and dysregulated in disease. Select Rab GTPases can also reciprocally modulate LRRK2 kinase activity through mechanisms that remain incompletely characterized. In this study, we examined how Rab29 and the PD-linked S71R-Rab32 mutant are able to stimulate LRRK2 kinase activity, focusing on their effects on LRRK2 dimerization. Using an overexpression system of different Rab GTPases along with two proximity biotinylation constructs of LRRK2 in HEK293T cells, we demonstrate that Rab29 enhances LRRK2 kinase activity without promoting its dimerization. In contrast, S71R-Rab32 activates LRRK2 by promoting dimerization, primarily via interactions with monomeric LRRK2. Our findings further reveal that disruption of the main dimerization interface of LRRK2 (COR-B) under S71R-Rab32 overexpression leads to partial reversal of its kinase-activating effects by reducing LRRK2 autophosphorylation but not downstream substrate phosphorylation. Together, these findings provide new mechanistic insight into Rab-mediated regulation of LRRK2, highlighting the heterogeneity in how distinct Rab GTPases influence its activity. Elucidating the mechanisms underlying LRRK2-Rab interactions will not only advance understanding of the pathobiology of Parkinson's disease but also provide a foundation for the development of targeted therapeutic strategies.



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Cold atmospheric plasma evokes breast cancer cell apoptosis through redox and matrix modulation

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We have recently demonstrated that cold atmospheric plasma (CAP) induced significant alterations in the expression of specific matrix effectors such as CD44 (a major cancer stem cell marker and receptor of hyaluronan), proteases and inflammatory mediators by breast tumor cells [1]. CAP is an ionized gas where the ions are close to room temperature and contains electrons, charged particles, reactive oxygen and nitrogen species (RONS). The objective of this project is to inquire into a CAP-based therapy for breast cancer subtypes that remain incurable. CAP treatment in both ER^+ and ER^- breast cancer cells induced drastic morphological changes and apoptosis (involving the mitochondrial pathway). However, the apoptosis rates were not identical probably indicating that the cellular antioxidant systems (for example thrioredoxin and glutaredoxin) differed between breast cancer cells of different ER profile and metastatic potential. The effect of CAP treatment on breast cancer cell viability in the absence or presence of RONS scavengers with different specificity revealed the prominent role of long-lived ROS, in particular H_2O_2, in the observed cytotoxicity of CAP. CAP-derived ROS induced the nuclear translocation of Nrf2 as well as its activation as evidenced by the increased transcription of anti-oxidant target-genes (HO-1, NQO1, GSR) indicating that tumor cells utilize Nrf2 to neutralize the CAP-induced cytotoxic effects. This was further confirmed by the observation that treatment with Nrf2 inhibitors prior to CAP enhanced breast cancer cell apoptosis. Moreover, CAP differentially regulated the oncogenic hyaluronan network (hyaluronan, HASes, HYALs, CD44s/v receptors) in breast tumor cells of different metastatic potential suggesting that CAP induced drastic structural and functional changes in the tumor microenvironment.
Overall, our data strongly support that CAP is a promising therapeutic for certain breast cancer subtypes given its selectivity against redox imbalance and tumor microenvironment reconstitution.

[1] Aggelopoulos et al., Frontiers in Oncology, 2022 (doi: 10.3389/fonc.2021.826865)



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TRAF3 as a regulator of breast cancer aggressiveness

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TRAF3 (TNF Receptor Associated Factor 3) is a regulator of NF-κB signaling, acting mainly as an inhibitor of the alternative NF-κB pathway through the interaction with other TRAF molecules and the downregulation of NIK (*MAP3K14*) kinase. While NF-κB has a well-established role in breast cancer development and progression, TRAF3 which acts as a ubiquitin-ligase in the NF-κB cascade is less studied in mammary carcinomas. Here by employing breast cancer cell lines stably expressing TRAF3 and mass spectrometry analysis for whole TRAF3 interactome in combination with functional assays and pathway signalling characterization, we sought to assess the role of TRAF3 in regulating aggressive traits of breast cancer cells. Our results indicate that TRAF3 protein overexpression affects colony formation of ER-positive cells in an inhibitory way. Proteome analysis for TRAF3 interactors identified multiple protein complexes related to cell cycle, DNA damage and apoptosis, pointing to a breast cancer cell proliferation regulatory role for TRAF3. Finally, immunoblotting on TRAF3 overexpressing breast cancer cell lines revealed BCL2 protein downregulation, a result relevant to apoptosis and proliferation. These findings further corroborate and expand our previous findings in breast cancer FFPE samples where TRAF3 protein expression presents a reverse correlation with histological grade and proliferation index (ki-67).



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Cancer Cell Metabolic Reprogramming Through Diet and Nutrition

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Background/Aim: Metabolic reprogramming, a key feature of cancer progression that enables tumor adaptation to stress and therapy, represents an ultra-novel target in anticancer research through dietary and pharmacological modulation. Dietary strategies such as caloric restriction (CR), intermittent fasting, and nutrient-specific modifications are emerging approaches to target oncogenic metabolism. This study aimed to summarize findings from in vitro models, in vivo animal studies, clinical observations, and multi-omics analyses to assess the potential of diet and pharmacological mimetics in influencing tumor metabolism and the tumor microenvironment (TME).

Materials and Methods: A systematic review was conducted on English-language studies investigating diet and nutrition-mediated metabolic reprogramming in cancer cells. Thirty peer-reviewed articles were identified and screened for relevance, recency, methodological rigor, and translational significance. The inclusion criteria focused on multi-omics analyses, mechanistic insights, and experimental or clinical evidence linking dietary interventions to metabolic pathways in cancer cells. Key findings were synthesized to outline current methodological approaches and to propose an integrative framework for handling diet-related multi-omics data in cancer metabolism research.

Results: Preclinical studies show that CR reduces tumor burden, inhibits IGF-1/mTOR signaling, and reverses obesity-driven transcriptomic signatures, including those related to epithelial-to-mesenchymal transition and hypoxia, while restoring immune pathways. *In vitro* evidence confirms that pharmacological inhibitors of IGF-1R and mTOR, especially when combined with autophagy blockade, synergistically decreasing cancer cell viability. Multi-omics analyses are undisputed technological facilitators revealing that both dietary restriction and pharmacological interventions remodel tumor microenvironment by altering immune infiltration and stromal interactions, and uncover metabolic dependencies—such as reliance on autophagy, amino acid metabolism, and redox balance—that represent actionable therapeutic vulnerabilities.

Conclusion: The findings position that dietary and pharmacological metabolic reprogramming as an innovative approach in anticancer research, capable of suppressing tumor growth and enhancing therapeutic response. Muti-omics-based experiments remain the driving force to keep unearthing such novel findings and help progress research on metabolic reprogramming of cancer cells.



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Functional implication of Clusterin isoforms in Osteosarcoma.

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Clusterin (CLU), a multifunctional extracellular chaperone, has been extensively investigated for its involvement in osteosarcoma, a bone malignancy associated with high morbidity rates. The secreted form of CLU (sCLU) has been shown to (among others) promote chemoresistance, metastasis and cell proliferation; consequently, has been proposed as a therapeutic target, or a potential biomarker in different cancers. In osteosarcoma the antisense oligonucleotide OGX-011, which targets CLU might serve as a promising adjuvant to established chemotherapeutic regimens, given its synergistic effects *in vivo*. However, several studies report contradictory findings, describing CLU as being pro-apoptotic, while in osteosarcoma the CLU-targeting combinatorial therapy has not yet been advanced in clinical trials. More recent research supports the existence of multiple CLU isoforms, derived from distinct mRNA transcripts, that have diverse and context-dependent roles in cellular stress responses. The specific functions and intracellular localization of these isoforms remain poorly characterized compared to sCLU, which may explain why CLU-targeted therapies have not yet reached clinical application. In this study we investigated in three osteosarcoma cell lines the existence of three different CLU mRNA transcripts, that may lead to distinct CLU isoforms. We also explored how genotoxic stress (induced by Doxorubicin, DXR) or proteotoxic stress (induced by Bortezomib, BTZ) affect the expression of these isoforms. Our results show the expression of all three mRNAs in these cell lines with differential expression patterns following DXR of BTZ administration. We also observed protein bands of unexpected band sizes in western blot analyses, suggesting the existence of novel or alternatively proteolytically processed isoforms. These findings provide a basis of the complex patterns of CLU expression and warrant further investigation to clarify their potential association with osteosarcoma progression.

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Establishment of prostate cancer and melanoma derived bone metastasis models in TgRANKL mice and evaluation of anti-resorptive drugs

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Receptor activator of nuclear factor-κB ligand (RANKL) plays a central role in osteoclastogenesis and bone resorption, and its inhibition by the monoclonal antibody denosumab is approved for the treatment of osteoporosis. However, denosumab does not cross-react with mouse RANKL and there are limited models to evaluate its *in vivo* efficacy. Bone metastasis can occur in various cancer types, including prostate cancer and melanoma, and recent studies have implicated RANKL in this process. Anti-resorptive agents, such as denosumab, which directly targets RANKL, and zoledronic acid, which induces osteoclast apoptosis, are currently under investigation in clinical trials for the prevention and treatment of bone metastases.

In this study, we developed bone metastasis models by injecting RM1-BM mouse prostate cancer cells and B16F10 mouse melanoma cells systemically into the caudal tail artery of syngeneic wild-type (WT) and osteoporotic TgRANKL mice, which overexpress the human RANKL gene. We aimed to assess the bone metastatic potential of cancer cells in TgRANKL mice and to investigate the effects of anti-resorptive drug treatment on the progression of bone metastases. Due to stable transduction of RM1-BM cells with the firefly luciferase gene, metastatic progression was monitored through *in vivo* bioluminescence imaging.

Our results showed that male TgRANKL mice exhibited accelerated progression of prostate cancer-induced bone metastases and more severe bone loss compared to their WT littermates. Prophylactic therapy with denosumab or zoledronic acid effectively prevented bone metastasis and inhibited osteolysis. Additionally, melanoma cells produced a higher metastatic burden across multiple tissues, including bones, in both male and female TgRANKL mice relative to WT controls. Prophylactic treatment with denosumab reduced bone loss and metastasis incidence in various tissues of TgRANKL mice. Overall, overexpression of RANKL in TgRANKL mice enhanced metastatic progression, whereas early administration of anti-resorptive agents demonstrated promising therapeutic benefits by reducing bone metastasis and mitigating osteolytic damage.

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Divergent Gastric T-cell Signatures in Autoimmune Gastritis and *Helicobacter pylori* Infection

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Introduction: Autoimmune gastritis (AIG) is a chronic inflammatory disorder characterized by autoreactive T cell responses against gastric parietal cell antigens. Similarly, infection with *Helicobacter pylori* (*H. pylori*) elicits pronounced gastric T cell activation. This study aimed to delineate mucosal T cell reactivity toward both self-antigens and *H. pylori* in patients with AIG, *H. pylori* infection, or their co-occurrence.

Materials & Methods: Symptomatic adults (n=40) from Italy were enrolled, comprising individuals with AIG in the absence of *H. pylori* infection (n=16, mean age 68 ±13 years), or AIG with *H. pylori* infection (n=15, 64 ±14 years), or patients with *H. pylori* infection without presence of AIG (n=9, 65 ±18 years). From each participant, six gastric biopsies and peripheral blood samples were collected. Mucosal T cells were isolated by Ficoll centrifugation, and proliferative responses to H^+/K^+-ATPase, intrinsic factor and *H. pylori* lysate were evaluated by [^{3}H]-thymidine incorporation. Cytokine secretion (IFN-γ, IL-17A, IL-4) was quantified using commercial ELISA.

Results: T cells from AIG patients exhibited proliferative responses to H^+/K^+-ATPase and/or intrinsic factor, but not to *H. pylori* antigens. In contrast, subjects with AIG and presence of *H. pylori* infection displayed reactivity to both self and microbial antigens, whereas *H. pylori*-infected individuals without AIG responded exclusively to bacterial antigens. Cytokine analysis revealed a predominant Th1/Th17 phenotype (IFN-γ and IL-17A production) across groups.

Conclusions: These findings demonstrate that autoreactive gastric T cell responses are specific to AIG, while *H. pylori* infection induces pathogen-restricted immunity. The co-occurrence of AIG and *H. pylori* infection may potentiate cross-reactive immune mechanisms, underscoring a potential immunopathogenic link between infection and autoimmunity. Ongoing research will focus on characterizing *H. pylori* isolates to elucidate putative strain-specific factors potentially influencing host immune modulation.



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Therapeutic Potential of Synthetic Neurosteroids in Psoriatic Inflammation

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Psoriasis is a chronic inflammatory skin disorder influenced by both genetic predisposition and environmental factors. However, its exact pathogenic mechanisms remain unclear. The disorder is marked by disrupted skin homeostasis and an exaggerated immune response, with significant infiltration of antigen-presenting cells that drive disease onset and progression. Previous clinical studies have linked reduced serum levels of the neurosteroid dehydroepiandrosterone (DHEA) with psoriasis and other dermatological conditions. However, therapeutic use of DHEA is limited due to its rapid metabolism into active sex hormones. BNN27, a synthetic DHEA analogue, which binds to TrkA and p75 receptors, exhibits strong anti-inflammatory and immunomodulatory properties, as well as a slower metabolic rate, suggesting enhanced therapeutic potential. Therefore, this study aimed to assess the effects of BNN27 in an imiquimod (IMQ)-induced murine model of psoriasis.

Methods: Wild-type male mice (8–12 weeks old) received daily topical IMQ application on the back and right ear to induce psoriasis-like lesions. BNN27 (10 mg/kg) and a TrkA inhibitor were administered intraperitoneally for eight days. Disease severity was evaluated using a modified Psoriasis Area and Severity Index (PASI), considering erythema, scaling, and skin thickness. At the end of the treatment, skin lesions were photographed, and blood and tissue samples were collected for analysis.

Results: BNN27 treatment, alone or combined with the TrkA inhibitor, significantly reduced PASI score compared with vehicle-treated mice. Histological analysis showed decreased epidermal thickness, normalized epidermal architecture, reduced dermal immune cell infiltration, and reappearance of the granular layer. However, flow cytometry revealed no major alterations in immune cell populations. Gene expression analysis showed reduced IL-10 mRNA levels following BNN27 treatment and with TrkA inhibition, alongside changes in AKT1 and AKT2 protein expression.

Conclusion: These results indicate that BNN27 may confer anti-inflammatory effects in psoriasis, warranting further research into its molecular mechanisms and therapeutic implications.



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The effect of simvastatin in the presence of hormones on functional properties and proteoglycan expression in triple negative breast cancer cells

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Breast cancer is the most common cancer type in women worldwide, with triple-negative breast cancer (TNBC), representing the most aggressive subtype, lacks expression of estrogen, progesterone, and HER2 receptors. Due to its poor prognosis and absence of targeted therapies, research has increasingly focused on identifying novel therapeutic strategies.

Our study investigated the effects of simvastatin on functional properties and proteoglycans' expression in TNBC cell lines. Concomitantly, the potential modulatory role of the steroid hormones 17β-estradiol and progesterone was assessed, either alone or in combination with simvastatin to partly mimic the state of the organism.

Simvastatin exhibited a similar cytostatic effect in both cell lines, MDA-MB-231 and Hs578T, with IC_{50} of 1.25 μM and 1.04 μM, respectively and reduced cell migration at higher concentrations, indicating inhibition of aggressiveness. Although steroid hormones enhanced migratory capacity, simvastatin abrogated this effect. The effect of simvastatin on spheroid formation was also studied. Morphological changes in spheroid structure and circularity were detected, suggesting reduced cell cohesion and potential phenotypic alteration.

Our findings also revealed differential regulation of proteoglycans' expression depending on simvastatin concentration and hormonal context. Decorin showed reduced mRNA expression but increased protein levels, possibly due to post-translational modifications or decreased degradation. Versican expression varied, no consistent pattern was observed and appeared to be hormone- and dose-dependent. Serglycin expression, while being decreased in the presence of estradiol, was found to be increased in almost all concentrations of simvastatin examined.

Overall, steroid hormones increased the aggressive phenotype of these TNBC cell lines and simvastatin largely reversed the hormonal activity affecting the expression of specific proteoglycans implicated in cell aggressiveness. Our findings suggest that the potential incorporation of simvastatin into chemotherapeutic regimens should be guided not only by the molecular characteristics of the tumor but also by the hormonal profile of the patients.



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microRNA profiling and cell-cell communication in 2D *vs.* 3D breast cancer cell models

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Cancer development and progression are profoundly influenced by dynamic interactions between cancer cells and their surrounding tumor microenvironment (TME). Within this context, the extracellular matrix (ECM) constitutes a structural scaffold, but also a functional regulator of cellular morphology and signaling. Breast cancer, the most prevalent malignancy among women and a leading cause of cancer-related mortality worldwide, exhibits extensive molecular and phenotypic heterogeneity. Our research group has recently demonstrated distinct expression patterns of ECM effectors between 2D and 3D breast cancer cell models, highlighting the biological relevance of 3D spheroids in studying breast cancer progression and therapeutic responses. Given the reciprocal interplay between microRNAs (miRNAs) and matrix effectors, along with the pivotal role of intercellular communication within the complex TME, this study aimed to investigate the miRNA expression profile, and extracellular vesicle (EV)-mediated communication in 2D and 3D breast cancer cell models. To this end, spheroids from three breast cancer cell lines with distinct estrogen receptor (ER) profiles, MCF-7 ($ERα^+$), MDA-MB-231 ($ERα^-/ERβ^+$), and shERβ MDA-MB-231 ($ERα^-/ERβ1^-$), were developed and subsequently characterized. Comparative analyses revealed distinct miRNA expression patterns associated with breast cancer progression in 3D culture systems of triple-negative and luminal A breast cancer cells. Among them, hsa-miR-145-5p exhibited the most pronounced alteration; therefore, transfections with pre-miR-145-5p were performed to assess its potential regulatory role on matrix- and adhesion/cytoskeleton-related targets. Additionally, scanning electron microscopy analysis indicated the presence of cell-cell communication structures, including small EVs and tunneling nanotubes, on the surface of both 2D cells and 3D spheroids, significantly suggesting the role of ERs status on intercellular communication dynamics. Collectively, these results highlight the culture-dependent regulation of miRNAs and EV-mediated intercellular signaling. Future studies will focus on the molecular characterization of EVs cargo to elucidate their specific roles in mediating cell-cell communication in breast cancer.

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Sulfated hyaluronan modulates extracellular matrix composition and miRNA expression in 3D breast cancer spheroids

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Breast cancer remains the most frequently diagnosed malignancy among women and a leading cause of cancer-related mortality worldwide. Cancer development and progression are directly influenced by the complex tumor microenvironment (TME), particularly by extracellular matrix (ECM) components. ECM is a highly organized 3D network of macromolecules, which not only provides structural support but also regulates cellular functional properties in both physiological and pathological conditions. Secreted and circulating microRNAs (miRNAs) play pivotal roles in cancer progression as they directly target ECM effectors, that affect cell-cell and cell-matrix communication through intricate regulatory networks. Hyaluronan (HA), the only non-sulfated glycosaminoglycan (GAG), is known to regulate breast cancer in a molecular-size-dependent way. Our research group has previously shown that chemically sulfated hyaluronan (sHA) regulates the functional properties and the gene expression profile of ECM effectors in breast cancer cells with different estrogen receptor status. While traditional 2D cell culture systems have contributed substantially in elucidating cancer biology, they present crucial limitations to mimic the complexity of *in vivo* TME. Recent research has shifted toward the development of advanced 3D cell culture models that more precisely replicate the dynamic cell-matrix-cell interactions of solid tumors. In this study, triple-negative breast cancer cell lines were treated with 50 kDa fragments of non-sulfated HA and sHA as to evaluate their effects on the expression of matrix macromolecules (i.e., metalloproteinases, proteoglycans, HA receptors) and selected miRNAs that play important roles in breast cancer progression. To this end, 3D spheroids from Hs578T and shERβ MDA-MB-231 breast cancer cells, were analyzed for differences in spheroid morphology and functional properties (i.e., growth rate, and dissemination capacity, EVs secretion). The expression levels of certain miRNAs associated with breast cancer progression, including miR-10b, miR-145, miR-200b, and let-7d are evaluated in both 2D and 3D breast cancer cell cultures. Moreover, the use of bioinformatics tools and databases, including Kaplan-Meier plotter, RAIN, and CancerMIRNome, contribute to further understand the importance of miRNAs in the progression of breast cancer. Collectively, these findings suggest new molecular insights on the role of sHA in matrix and miRNA regulation during breast cancer progression, potentially guiding the development of novel matrix-based therapeutic strategies targeting aggressive breast cancer subtypes.

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Wisp1 matricellular protein enhances the resistance of various cancer cells against the chemotherapeutic drug doxorubicin through Nrf2 activation

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Wisp1 is a secreted matricellular protein, which plays a pivotal role in several cell responses, including differentiation, proliferation, migration, and survival, highly expressed in various cancers, such as breast and prostate. Doxorubicin is one of the most potent anticancer drugs, exerting its antitumor effects through intercalation into DNA as well as through production of reactive oxygen species (ROS) that cause oxidative stress and apoptosis. However, it has been shown that doxorubicin can induce drug resistance in cancer cells, resulting in poor patient prognosis and survival.

In this study, we investigated how doxorubicin can induce drug resistance in cancer cells as well as the involvement of Wisp1 in this process. Breast (MCF7, MDA-MB-231) and prostate (PC-3) cancer cells were treated with doxorubicin in the absence or presence of Nrf2 transcription factor inhibitors (brusatol, chrysin) and the ROS scavenger NAC. A reduced viability of cells was observed in the presence of Nrf2 inhibitors or NAC, indicating that Nrf2 and ROS are implicated in the doxorubicin resistance of cancer cells. In fact, doxorubicin was able to induce (i) ROS production, (ii) the nuclear translocation and activation of Nrf2, and (iii) the expression of antioxidant enzymes. When cancer cells were treated with doxorubicin in the presence of Wisp1, an enhanced viability of cells was observed, which was reversed in the presence of Nrf2 inhibitors, indicating that Nrf2 was implicated in the Wisp1's enhancement effect. Notably, Wisp1 was able to induce Nrf2 nuclear translocation and activation, and subsequent expression of antioxidant enzymes.

These results show that the resistance of cancer cells against doxorubicin and its enhancement by Wisp1 are mediated by their stimulatory effect on Nrf2 nuclear translocation and activation. These findings showcase an oncogenic role of Wisp1 and highlight the Nrf2 pathway as a possible therapeutic target in combination with doxorubicin chemotherapy.



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Urolithin A mediated-mitophagy restores mitochondrial and lysosomal homeostasis in a *C. elegans* model of Werner Syndrome

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Werner Syndrome (WS), also known as adult progeria, is a rare autosomal recessive disorder characterized by mutations in the *wrn* gene, which encodes the Werner (WRN) DNA helicase. Individuals with WS exhibit signs of accelerated aging, such as graying hair, cataracts, skin atrophy, osteoporosis, type 2 diabetes, and an increased risk of cancer. The loss of WRN function results in genomic instability, impaired DNA repair, and metabolic dysfunction. Werner Syndrome mechanistically recapitulates all ten hallmarks of aging. Recent studies suggest that mitochondrial damage is a central feature in WS pathogenesis. Defective mitochondria in WS not only produce reduced levels of ATP but also release pro-apoptotic and inflammatory factors, and generate elevated levels of reactive oxygen species (ROS), leading to genomic instability and cell death. Here, we investigated mitochondrial impairment as a key driver of WS and explored the therapeutic potential of Urolithin A (UA), a mitophagy-inducing agent. Utilizing *Caenorhabditis elegans* as a model organism, we found that UA treatment mitigated DNA damage, improved mitochondrial homeostasis, and significantly extended the lifespan of WRN-deficient nematodes in a PINK-1 dependent manner. Proteomic analysis further revealed that UA supplementation enhanced proteome integrity, activating several novel pathways, including lysosomal and peroxisomal functions, underscoring UA's broad impact on cellular homeostasis. Notably, we report unpreceded lysosomal dysfunction in WS nematodes which was ammeliorated by the UA supplementation. Our findings highlight UA as a promising therapeutic candidate for WS and related progeroid syndromes. Taken together, our study underscores the critical role of mitochondrial and lysosomal integrity in WS pathogenesis and opens new avenues for targeted therapeutic strategies, warranting further investigation and clinical validation of UA.



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Pancreatic Cancer-Associated Depression (PCAD) is linked to adult neurogenesis impairment

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Pancreatic cancer (PC) is a highly aggressive cancer and often linked to major depression (PCAD) before diagnosis. Our study investigates the mechanism behind this link by examining how PC impacts the brain to induce depression. For this purpose, we conducted behavioral tests on a PC mouse model created by orthotopically injecting human Panc-1 cells into NOD-SCID mice. These mice exhibited a depressive-like phenotype compared to sham-operated controls. HPLC analysis of their brains revealed significantly altered serotonin levels, also evident in a genetic PC mouse model (Pdx1Cre-AKras^{G12D}) that mimics human PC development via pancreas-specific overexpression of the mutated Kras^{G12D} oncoprotein. Both models showed impaired adult hippocampal neurogenesis (AN), demonstrated by reduced DCX^{+} cells in the dentate gyrus. Additionally, hippocampal neural stem cell (NSC) pools were reduced at later PC stages, as indicated by decreased GFAP^{+}/radial glia-like NSCs. To evaluate the involvement of systemic factors on AN impairment during PC progression, we cultured wild-type hippocampal NSCs with serum from the PC mouse models. Exposure to these sera reduced survival as revealed by TUNEL analysis. A Luminex-based panel measured multiple cytokines in serum from PC mice, revealing distinct increases in pro-inflammatory factors across the two PC models. IL-6, MCP-1, and G-CSF were elevated in NOD-SCID mice with PC, while TNFa, IL-1b, IL-1a, and G-CSF were higher in Pdx1Cre-AKrasG12D mice. Transcriptomic analysis of the Pdx1Cre-AKras^{G12D} hippocampi revealed an increased mRNA profile of factors related to neuroinflammation, known to decrease aNSCs survival, such as IL-1a, IL-1b, TNFa, and C3. These findings suggest an adult neurogenesis-related mechanism for PCAD, highlighting specific pro-inflammatory factors as key players in this process.



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RNase κ: A Key Regulator of Dengue Virus Proliferation and Its Implications in Cellular Homeostasis

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RNase κ is a highly conserved endoribonuclease found across nearly all metazoans and has been implicated in diverse cellular processes. Recent research identified RNase κ as a host factor required for the entry of several viruses, including Dengue virus (DENV), via clathrin-mediated endocytosis. However, its potential roles in later stages of viral infection remain unexplored. In this study, we investigated the contribution of RNase κ to DENV replication beyond viral entry. To this end, we performed stable silencing of RNase κ in Huh7 cells and then infected cells with DENV-2 for 72 h or bypassed viral entry by electroporating cells with the virus subgenomic replicon or full-length genome. While infection assays revealed that RNase κ knockdown modestly enhanced viral replication and virus-released infectivity, electroporation experiments demonstrated a much stronger effect, for both subgenomic replicon and full-length viral sequence. These findings suggest for the first time that RNase κ restricts DENV life cycle at stages. Consistently, DENV infection, in turn, led to downregulation of RNase κ expression at RNA and protein levels, highlighting a dynamic virus–host interaction whereby the virus modulates RNase κ to promote its own propagation. Given the established links of RNase κ to cell survival, we further examined its role in DENV-induced cell death by measuring ATP levels, cell proliferation and the expression of innate immune genes that regulate cell fate, in shControl and shRNase κ cells. To specifically address, the implication of RNase κ in DENV-induced apoptosis, we analyzed caspase and BCL-2 family members, alongside Annexin V/PI assay to distinguish apoptotic populations. Collectively, these results demonstrate that RNase κ affects negatively DENV replication and is implicated in cellular pathways that are crucial for the viral life cycle, positioning it as a key regulator of the tripartite relationship between RNase κ, DENV, and cell homeostasis.



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Dyrk1B kinase positively regulates SOD1 expression in SOD1^{G93A} mice

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We have previously demonstrated that Dyrk1B kinase regulates ventral progenitors and the generation and survival of spinal motor neurons (SpMNs) in the embryonic chick spinal cord, affecting specifically the LMCm motor column, which innervates ventrally the muscles of the limbs via Shh/Gli pathway. Dyrk1B suppresses Shh/Gli signaling, while its pharmacological inhibition by AZ191, a specific Dyrk1B inhibitor, up-regulates Shh/Gli signalling resulting in increased number of ventral progenitors and SpMNs. Here, we used SOD1^{G93A} mice to validate Dyrk1B kinase as a novel pharmacological target in SOD1-mediated amyotrophic lateral sclerosis (ALS). We found that embryonic spinal cord of SOD1^{G93A} mice exhibits increased Dyrk1B expression, reduced Shh/Gli signalling and neurodevelopmental deficits characterized by reduced numbers of ventral progenitors p3, pMN and p2 by 1.50-fold, 1.43-fold and 1.30-fold respectively and of SpMNs by 1.42-fold. These data reveal that the loss of SpMNs begins from the embryonic life of SOD1^{G93A} mice. Moreover, E12.5 SOD1^{G93A} SpMNs exhibit fragmented and shorter axons by 1.53-fold, increased apoptosis by 5.53-fold, increased SOD1 protein expression by 2.0-fold and increased protein aggregates by 1.77-fold. Furthermore, E12.5 SOD1^{G93A} SpMNs display an intense accumulation of ubiquitinated proteins by 1.82-fold and increased autophagy by 2.32-fold. Pharmacological inhibition of Dyrk1B in SOD1^{G93A} SpMNs, by using AZ191, restores axonal phenotype, reduces apoptosis at the normal levels, restores proteasomal dysfunction and decreases endogenous SOD1 protein expression by 1.69-fold. Dyrk1B function in SOD1 protein expression was further investigated in Neuro2A-Dyrk1B cells, demonstrating that Dyrk1B overexpression increases endogenous SOD1 protein expression, while pharmacological inhibition of Dyrk1B in Neuro2A-Dyrk1B cells restores endogenous SOD1 protein expression at the basal levels. Collectively these data reveal that Dyrk1B kinase positively regulates SOD1 protein expression in SOD1^{G93A} SpMNs and Neuro2A cells and strengthen the therapeutic potential of AZ191 inhibitor in SOD1-mediated neuropathology by conferring neuroprotection, restoring proteostasis and by decreasing endogenous SOD1 protein expression.



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Correlation of Levels of Factor VII with Mutation Arg353Gln in F7 Gene In Patients with Liver Disease

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The present study aimed to investigate the correlation of decreased levels of coagulation factor VII with the Single Nucleotide Polymorphism (SNP) Arg353Gln in factor VII gene in patients with liver disease. **Patients – Materials and Methods:** For this study 40 samples from patients with liver disease were used and 19 donors as control group. Firstly, the basic hemostasis panel was performed (PT sec, PT INR, aPTT, Fibronogen, D-Dimers) and after factor VII was measured in analyzer Stago Star Max[3]®. In addition, the detection of the mutation Arg353Gln was performed using the Real Time PCR method. The data was processed with SPSS 29.0. **Results:** In 62% of patients this mutation was not detected, however 35% of patients were heterozygous and 3% were homozygous. Among patients with liver disease, the proportion of abnormal Factor VII levels ranged from 20% to 100%. Individuals without Factor VII mutation, whether from the patient or control group, exhibited normal levels. Liver disease patients identified as heterozygous showed 42% abnormal level, compared to approximately 33% in the controls. Also, homozygous individuals exhibited 100% abnormal levels, indicating that homozygosity for this specific mutation has a profound effect on Factor VII levels. Examining the correlation between the polymorphism and Factor VII levels, it was found that in the control group, the correlation was statistically significant ($p=0.0005$). However, in patients with liver disease, due to the multifactorial nature of the condition and the limited sample size, there wasn't a statistically significant correlation. **Conclusions:** According to the results of this study, a correlation of Factor VII levels in liver disease and the mutation since the combination of these two negatively affects the factor VII levels. Nevertheless, further investigations in patients with liver disease are necessary, because there isn't a statistically significant between the decreased levels of factor VII and mutation Arg353Gln.



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The proteoglycan serglycin acts as a crucial regulator of the unfolded protein response (UPR) pathway in glioblastoma

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Serglycin (SRGN), the only known intracellular proteoglycan (PG), was originally discovered in hematopoietic cells, where it facilitates the formation of secretory granules and the regulated release of their components, such as proteases and inflammatory mediators, into the extracellular matrix (ECM). Simultaneously, recent studies, including results from our group, have revealed the oncogenic role of SRGN in several malignancies, such as breast cancer, multiple myeloma, and glioblastoma multiforme (GBM). Endoplasmic reticulum (ER) stress, characterized by the accumulation of misfolded or unfolded proteins, activates the unfolded protein response (UPR), an adaptive mechanism that restores proteostasis. In this study, we sought to elucidate the role of SRGN in regulating the UPR. To address this, we utilized two GBM cell lines: one stably expressing SRGN (LN-18^{shSCR}), and another with suppressed SRGN expression (LN-18^{shSRGN}). ER stress was induced using the N-glycosylation inhibitor tunicamycin (TM). Our results revealed that TM suppressed proliferation and migration in both cell lines. However, LN-18^{shSRGN} cells exhibited higher sensitivity to lower TM concentrations, resulting in increased cell death. UPR markers showed decreased activation in LN-18^{shSRGN} cells compared to LN-18^{shSCR} cells. Analysis of caspase-3 and PARP-1 cleavage revealed increased pro-apoptotic activity in LN-18^{shSRGN} cells and reduced levels of anti-apoptotic mediators. Alongside these observations, expression of inflammatory receptors and NF-κB activity were diminished in LN-18^{shSRGN} cells, suggesting a potential crosstalk between UPR, inflammation, and cell survival pathways modulated by SRGN. Transient knockdown of SRGN in another GBM cell line, U251-MG, confirmed the involvement of SRGN in the crosstalk between UPR, inflammation, and cell survival pathways. Collectively, our findings position SRGN as a central regulator of the UPR in GBM, linking ER stress response, apoptosis, and inflammatory signaling to tumor cell survival.

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Serglycin affects unfolded protein response (UPR) pathway and the ubiquitin-proteasome system (UPS) in multiple myeloma

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Serglycin (SRGN), the only known intracellular proteoglycan, was originally characterized for its role in secretory granule maturation and regulation of cargo bioavailability. Recent studies have identified SRGN as a multifunctional proteoglycan that drives oncogenic signaling and modulates tumor cell phenotypes. Our previous work demonstrated that elevated SRGN expression correlates with increased tumor aggressiveness across several malignancies, including breast cancer, multiple myeloma, and glioblastoma multiforme. Given that many malignancies exhibit increased protein secretion and turnover, tumor cells rely heavily on adaptive mechanisms, such as the unfolded protein response (UPR) and the ubiquitin-proteasome system (UPS), to cope with proteotoxic stress. In this study, we investigated the role of SRGN in regulating these proteostatic mechanisms in multiple myeloma. Using U266 and H929 multiple myeloma cell lines, SRGN expression was silenced via siRNA, and the effects on key UPR and UPS components were evaluated. Basal UPR marker expression differed between the two cell lines. Moreover, SRGN knockdown induced distinct changes in the expression and activation of UPR and UPS components, suggesting a cell line–dependent regulatory mechanism. Collectively, these findings suggest a role for SRGN in multiple myeloma cell proteostasis through selective regulation of the UPR and UPS pathways. This underscores the contextual complexity of SRGN function and highlights its potential as a therapeutic target for disrupting adaptive proteostatic networks in malignant plasma cells.

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Macropinocytosis Regulates Amyloid-β Uptake and Clearance Across the Blood–Brain Barrier and Modulates Plaque Deposition in Alzheimer's Models

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Deficient clearance of amyloid-β (Aβ) across the blood–brain barrier (BBB) is a central pathogenic feature of Alzheimer's disease (AD) [1]. Although receptor-mediated transcytosis pathways are well established, such as the LRP1-mediated pathway [2], the role of alternative endocytic routes in Aβ uptake and clearance remains poorly defined. In this study, we investigated the contribution of macropinocytosis and dynamin-dependent endocytosis to Aβ endocytosis using human brain endothelial cells, as *in vitro* model, and the 5xFAD transgenic mouse model of AD, for in vivo validation. Selective inhibitors (EIPA, a macropinocytosis blocker; Dynasore, a dynamin-dependent endocytosis inhibitor) and siRNA-mediated knockdowns were applied to map Aβ1-42 internalization routes. Uptake was assessed by confocal microscopy using transferrin and the fluid-phase marker Dextran70, while amyloid burden in vivo was quantified by immunofluorescence and Western blotting. Interestingly, brain endothelial cells internalized Aβ through at least two distinct mechanisms: macropinocytosis and clathrin-mediated endocytosis. Inhibition of macropinocytosis with EIPA or siRNA-mediated knockdown of Rac1 and Cdc42, markedly reduced Aβ internalization *in vitro*. *In vivo* experiments in 5XFAD mice (a model of AD) demonstrated that chronic administration of endocytosis inhibitors over 1.5 months is accompanied by increased Aβ levels in the brain, highlighting the role of endocytic pathways in Aβ brain accumulation. Conversely, dynasore treatment produced a modest reduction in amyloid burden, consistent with decreased APP internalization and subsequent Aβ generation. These findings demonstrate that macropinocytosis is a key pathway for Aβ clearance at the BBB and reveal a complex interplay between multiple endocytic mechanisms in regulating Aβ trafficking and deposition. Targeting the balance between macropinocytic and receptor-mediated endocytosis may offer novel therapeutic opportunities to enhance Aβ clearance and mitigate both vascular and parenchymal amyloid pathology in AD.

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Alterations of Long COVID-isolated Extracellular Vesicle Cargo Reveals Similarities to Alzheimer-Like Pathology

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Long COVID (LC), following SARS-CoV-2 infection, often presents with cognitive decline and neuroinflammatory changes resembling those observed in Alzheimer's disease (AD) and metal-induced neurotoxicity. These conditions share molecular features such as oxidative stress and glial activation, suggesting that microglia play a central role in neuronal vulnerability. Extracellular vesicles (EVs) have emerged as key mediators of intercellular communication and potential carriers of neurodegenerative biomarkers. Whether microglial stress triggered by SARS-CoV-2 exposure remodels EV composition and contributes to Tau pathology, similarly to other inducers of oxidative/inflammatory stress, such as heavy metals, remains unclear. To address this question, serum-derived EVs from LC and AD patients were enriched for neuronal ($L1CAM^+$) and small EV ($CD63^+$) subpopulations to assess neurodegeneration-associated cargo, including total and phospho-Tau and glial markers. In parallel, human microglial (HMC3) and neuronal (SH-SY5Y) cell models were used to examine stress-induced vesicular crosstalk. Inorganic iron was applied to HMC3 to induce metal-driven oxidative stress and generate microglia-derived EVs, while SARS-CoV-2 virus-like particles (VLPs) were incubated with SH-SY5Y monocultures and SH-SY5Y–HMC3 co-cultures to mimic viral exposure. The development of an optimized EV isolation protocol enabled consistent biochemical and biophysical profiling. Comparative analyses of EV profiles between LC and AD clinical samples revealed condition-dependent alterations in vesicle cargo, particularly in glial proteins and phospho-Tau species. SARS-CoV-2 VLP exposure increased neuronal susceptibility *in vitro*, an effect amplified in the presence of microglia. Iron supplementation altered microglial responses and stressed microglial EV cargo elicited neuronal effects comparable to those induced by VLPs. *In vitro* findings may point to a converging mechanism through which iron imbalance and virus-induced stress contribute to Tau-associated pathways. Overall, integration of patient-derived and cellular data highlights EVs as dynamic mediators of glia–neuron communication, suggesting that SARS-CoV-2-mediated changes in EV cargo presented in Long COVID syndrome could trigger AD-related neurodegenerative mechanisms.

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Disruption of the mitochondrial homeostasis after prolonged adrenergic stress primes the innate immune response in salivary gland epithelium

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Stress is a physiological response of the body to a challenging or a difficult circumstance. When the duration of stress is long, the concept of stress is defined as chronic stress, characterized amongst others, by prolonged activation of the sympathetic nervous system and secretion of the hormones epinephrine and norepinephrine. Chronic stress is an important regulator of immunity; however, the molecular mechanisms are poorly understood. Mitochondrial damage is connected to innate immunity since mitochondrial stress can lead to the release of mtDNA in the cytosol and trigger type I interferon (IFN) response. Type I IFN signature is a hallmark of Sjögren's disease (SjD) and other autoimmune diseases such as Systemic Lupus Erythematosus. SjD is a rather common autoimmune disease, the most prominent feature of which are the salivary gland infiltrations by T and B lymphocytes *in situ*. Salivary gland epithelial cells (SGEC) are active participants in the induction and perpetuation of the inflammatory process and their phenotype in SjD is mainly characterized by increased adrenergic signaling resembling chronic stress conditions. Thus, SjD-derived SGEC provide a robust and credible model to characterize cellular responses triggered by chronic stress. Here we show that prolonged adrenergic stress (PAS) elicited by epinephrine, affects mitochondria homeostasis and engages type I IFN response conferring autoimmune milieu. We found that PAS reprograms mitochondria in SjD-derived SGEC but not in control SGEC, as mitochondrial proteins show non-canonical response to it. Furthermore, PAS induces mitochondrial stress as seen by the escape of mtDNA in the cytosol, which was validated by real-time PCR and immunofluorescence with anti-TOM20 and anti-DNA. Moreover, PAS increases the expression of interferon-beta 1 (IFNb1) as well as of interferon-stimulated genes, such as OAS1 and OAS3. These data demonstrate that PAS compromises mitochondria machinery and primes innate immune responses in SjD-derived SGEC.



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Dysregulated sympathetic nervous system shapes peripheral immune responses in Systemic Lupus Erythematosus potentially contributing to flares

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The neuro-immune crosstalk contributes to the pathogenesis and progression of autoimmune diseases like Systemic Lupus Erythematosus (SLE). Disruptions in sympathetic nervous system (SNS) activity, particularly during stress observed in SLE patients, may modulate peripheral inflammatory response by altering the release of norepinephrine (NE) in peripheral tissues, contributing to immune imbalance in SLE. To this end, we compared to healthy individuals, whole-blood transcriptomic data of SLE patients and found an enrichment of SNS/NE-related pathways. In the lupus-prone NZBW/F1 mouse model, a similar enrichment was detected in the kidney transcriptomes of pre-diseased (3 months-old) mice but not in the diseased (6 month-old), where NE levels - measured by HPLC - showed a 1.4-fold decrease in the kidney and a 2.5-fold decrease in the spleen. Exploring its signaling pathway, expression of adrenergic receptors was predominantly reduced on immune cells isolated from the spleen and kidney of diseased mice, with B cells, tissue-resident macrophages, neutrophils and Ly6C^{high} monocytes displaying decreased expression for a1 and b2 receptors. A lower adrenergic receptor expression on dendritic cells was only observed in the kidney, while splenic Ly6C^{low} monocytes exhibited a significant increase in a1-adrenergic receptor expression. Ongoing in-vitro and in-vivo assays will further elucidate these mechanisms. We propose that increased NE levels at early disease stages may facilitate the switching to the autoimmune phenotype of lupus B cells by enhancing their activation, plasmablast/plasma-cell development, and promote the pro-inflammatory phenotype of other immune cells. Acute or chronic stress and anxiety may further elevate NE levels, contributing to immune dysregulation and fueling flares in SLE.



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Differential induction of MAPK signaling pathways in gastric epithelial cell lines following *Helicobacter pylori* infection.

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Helicobacter pylori (Hp) infection is major etiological factor for the induction of chronic gastritis and peptic ulcer disease and remains the primary risk factor for gastric adenocarcinoma development, augmented by the activity of its bacterial CagA oncoprotein. Previous work in our laboratory utilizing 24h *Hp*-infected gastric epithelial cell lines and *in vivo* mouse models of *Hp* infection has demonstrated that the mitogen-activated protein kinase (MAPK) pathway and more specifically, activated ERK1/2 and JNK, critically regulate the induction of matrix metalloproteinases-3 (MMP-3) and MMP-9, involved in the extracellular matrix remodeling and induction of Epithelial-to-Mesenchymal-Transition (EMT) in the gastric lamina propria. Here, we aimed to investigate the early induction and temporal regulation of MAPK signaling pathways following *H. pylori* infection in gastric epithelial cell lines. Gastric cell lines (GES-1, MKN45, AGS) were infected with an isogenic mutant of P12 strain expressing a more virulent isoform of CagA protein, at MOI:100. Phosphorylated and total forms of ERK1/2 and JNK were analyzed by Western blotting at four different incubation time points (0.5h, 1h, 2h, 4h). No induction of ERK1/2 and JNK was observed in GES-1, a SV40 transformed human fetal gastric epithelial cell line. On the contrary, both pathways were found activated at 1 and 2 hours post-infection in MKN45 and AGS respectively, both cell lines derived from gastric adenocarcinoma female patients. Our primary results indicate differential response among cell lines of varying differentiation state. Further work, with the use of chemical inhibitors will assess MAPK signaling and crosstalk during *Hp* infection, across the EMT differentiation axis. Understanding these signaling mechanisms can provide insights into *Hp*-host interactions and identify potential molecular targets for therapeutic intervention.



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The Role of LSD1/EMMPRIN Axis in HCV-associated Hepatocellular Carcinoma and Metastasis

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Hepatitis C virus (HCV) chronic infection leads to liver cirrhosis and hepatocellular carcinoma (HCC), a highly metastatic tumour. The virus interacts with host proteins, leaving epigenetic and metabolic footprints. HCV regulates the epigenetic factor lysine-specific demethylase (LSD1) and fosters activation of metastatic pathways, such as the increase in matrix metalloproteinases (MMPs) regulated by the extracellular matrix (ECM) metalloproteinase inducer (EMMPRIN). We aimed to investigate whether HCV modulates EMMPRIN and assess the existence of a putative LSD1/EMMPRIN regulatory axis that could promote ECM degradation and increase metastatic potential of hepatoma cells during long-term viral infection. We used immunoblotting to study EMMPRIN protein expression upon overexpression and knockdown or functional inhibition of LSD1 in Huh7.5 cells during long-term HCV infection and expression of viral proteins HCV core and NS5A. EMMPRIN function was assessed by evaluating its hyperglycosylation status and targets involved in ECM degradation by zymography and biochemical analysis. Wound healing and invasion assays determined the ablility of LSD1/EMMPRIN to promote metastasis. A tightly regulated, wave-like fluctuation of hyperglycosylated EMMPRIN was observed in infected HCC cells over a twenty-day HCV infection course. Deglycosylation of infected cell extracts revealed that HCV regulated both expression and hyperglycosylation of EMMPRIN. Overexpression of LSD1 increased total protein, as well as the hyperglycosylated form of EMMPRIN, in contrast to silencing or pharmacological inhibition of LSD1, thereby altering expression and activity of target MMPs and collagen content. LSD1/EMMPRIN axis increased cell motility and invasion. Our data suggest a strong effect of *in vitro* long-term HCV infection on the expression and functionality of EMMPRIN, which may be due to differential expression of major HCV proteins throughout the viral life cycle. Furthermore, we established the existence of an LSD1-EMMPRIN regulatory axis that influences EMMPRIN-dependent regulation of ECM degradation, enhancing the metastatic potential of cells from within the hepatoma milieu.

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Chemoresistance-related signatures in a doxorubicin-resistant cell model of osteosarcoma

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Chemoresistance, the tolerance of tumor cells to therapeutic agents, is one of the most significant challenges of cancer treatment. Osteosarcoma (OS) is a malignant tumor mostly occurring in long bones; it is associated with the pathogenesis of mesenchymal cells, and it predominantly affects young teenagers. Notably, the etiology of the disease along with development of resistance to treatment remain largely unexplained. Here, we studied the transcriptomic and proteomic signatures of a doxorubicin (DXR)-resistant OS cell model to identify *a*. possible mechanisms and/or biomarkers of resistance development, and *b*. druggable targets. The DXR-resistant OS cell model was developed by the exposure of two different OS cell lines bearing distinct genetic backgrounds, namely U2OS, and KHOS cell lines, to gradually increasing concentrations of DXR; we thereby produced different generations (R1, R2, and R3) of OS cells being resistant to increasing doses of the drug. Bioinformatics analysis revealed the gene and protein expression signatures among the cell lines and within the distinct DXR-resistant cell lines. Comparative analysis of the combined *-omics* datasets highlighted the common patterns and led to the selection of five possible targets of chemoresistance (SRI, Rad50, FGF2, NUDCD2 and NUFIP2). Validation of all 5 targets in the cell lines confirmed the overexpression patterns during the progression of chemoresistance. Our ongoing downstream bioinformatic studies aim to identify known drugs that target the deregulated proteins; these drugs will be studied for their potential activity against chemoresistance (repurposing). Validating these data in animal models and in the clinic, along with a deeper investigation of candidate biomarkers and potential drug targets, will further enhance the translational aspect of this study.

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Hyperosmotic stress-induced overexpression of type II arginase in nucleus pulposus intervertebral disc cells

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Intervertebral disc (IVD) degeneration has been considered the major cause of low back pain, which imposes a great socioeconomic burden worldwide. IVD cells -especially of the nucleus pulposus (NP) - are constantly exposed to a hyperosmotic extracellular milieu in comparison to cells comprising most other tissues of the body. Using a genome array technology to assess high osmolality-induced transcriptional changes in NP IVD cells, >100 and 200 genes have been found to be differentially expressed after a 5- and 24-h hyperosmotic treatment, respectively [J Cell Physiol. 2015 Dec;230(12):3037-48]. Functional annotation of upregulated genes classified ARG2 within the *positive regulation of cellular senescence* category. Aim of the current study was to explore ARG2 implication in the osmo-regulatory response, as well as in senescence induction/manifestation in NP IVD cells. Based on our findings, under hyperosmotic conditions, mitochondrial function, metabolic activity, proliferative potential and viability of NP IVD cells were impaired. ARG2 expression was increased at the transcriptional and protein levels in a time- and dose-dependent, but tissue- and species-independent manner. Interestingly, ARG2 overexpression was long-lasting since mRNA and protein levels remained high even days post-hyperosmotic treatment. Moreover, hypo-osmotic stress also resulted in ARG2 overexpression. Reactive oxygen species were found to participate to an extent in the high osmolality-induced ARG2 upregulation. Competitive inhibition of ARG2 partly abrogated NP IVD cells' proliferative arrest and apoptosis. Finally, senescent cells were characterized by similar ARG2 levels compared to their young counterparts, more likely implying a a causative role of the protein in senescence induction. Given that IVD cells are responsible for the maintenance of the tissue's homeostasis, understanding their physiology taking into account parameters met in their microenvironment *in vivo* is of outmost importance to design efficient therapeutic approaches for disc degenerative disorders.

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Correlation of AHSP gene variants, detected by HRMA, with distinct haematological phenotype of alpha thalassaemia carriers with identical HBA genotype

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AHSP (Alpha-Hemoglobin Stabilizing Protein), a small protein functioning as a "molecular chaperone", promotes the correct folding and stabilization of α-globin and facilitates its complex formation with beta globin and its incorporation into the hemoglobin tetrameric complex. AHSP-αHb complex prevents precipitation and oxidative damage associated with excess free α-globin. Given the role of AHSP as a modifier of thalassaemic syndromes phenotype, the gene was screened in α-thalassemia heterozygotes with identical HBA genotype but distinct haematological phenotypes.

20 heterozygotes for the αPolyA1 point variant (NM_000558.5(HBA2:c.*+94A>G)) were selected and categorized into 5 subgroups based on their erythrocyte indices MCV and MCH values. AHSP gene scanning was performed by High Resolution Melting Analysis (HRMA), in genomic DNA samples from peripheral blood. Specific primer pairs were designed by Primer3 software, covering overlapping regions of AHSP gene (NG_046852.1). The melting profile analysis performed revealed three variants. The variants 5258G>A (Oct-1 transcription factor binding region) and 5762G>T (exonIII) were detected in samples from different subgroups. The variant 5762G>T has been characterized as non-pathogenic in other populations. Interestingly, the variant 5214G>A, located near the binding site of the transcription factor Oct-1, was detected in four samples, three of which had a differentiated hematological phenotype (increased MCV and MCH values compared to the typical ones).

Overall, analysis of larger sample size is required for correlation of specific AHSP gene variants with the regulation of hemoglobin production in the erythrocyte. Still, in the era of high throughput sequencing methodologies, the HRM genomic region scanning methodology enables the simultaneous analysis of a large number of samples with speed, sensitivity, repeatability and quite low cost. The establishment of a rapid, automated and highly sensitive AHSP analysis process is expected to contribute to diagnosis, personalized genetic counseling and the possible discovery of therapeutic approaches for thalassemia patients, especially in the thalassemia intermedia spectrum.



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Hepatitis C Virus-directed exoPD-L1 as a key modulator of host adaptive immunity

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Chronic HCV patients develop liver disease leading to cirrhosis and hepatocellular carcinoma. Direct acting antivirals (DAAs) eliminate the virus, but the adaptive immune response does not fully recover. HCV exploits exosomal cargo to establish viral infection, enhance viral replication and evade the immune response. Exosomes mediate immunosuppression by delivering immunoregulatory molecules like Programmed death-ligand 1 (PD-L1) to immune cells. We aimed to confirm the presence of PD-L1 in exosomes isolated from the *in vitro* HCV cell culture system before and after treatment with DAAs and elucidate its role on adaptive immunity. For this purpose, Huh7.5 cells were infected with HCV and treated with DAAs. Exosomes characterization was performed by Nanoparticle Tracking Analysis and immunoblotting against specific extracellular vesicles (EVs) markers. Jurkat T-cells were incubated either with PMA/ionomycin or with isolated hepatic exosomes and specific signaling molecules and cytokines were used to measure T-cell activation by Western blot and RT-qPCR, respectively. The expression of PD-L1 in the isolated exosomes was investigated by Western blotting and ELISA. FACS analysis evaluated the presence of PD1 and exoPD-L1 under certain conditions. We observed a mild induction of PD1 on the surface of T-cells in the presence of hepatic exosomes that contained PD-L1 before and after DAA treatment. Specificity of this response was confirmed by the positive reaction of PMA/ionomycin. Colocalisation of exoPD-L1 with CD4+T–cells demonstrated the formation of PD1/PD-L1 complex. MAPK-ERK phosphorylation was investigated as a possible signal for PD1/exoPD-L1 complex-mediated regulation of IL-2. We confirmed that exosomes mediate the transfer and localization of hepatic exoPD-L1 onto T-cells after DAA treatment. ERK-mediated IL-2 reduction could signify a blockade of T-cell proliferation and function through formation of the PD1/exoPD-L1 complex. We propose that virus-driven loading of specific factors in the exosomal cargo, could sustain deregulation of the host immune response long after HCV eradication.



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Modeling Vascular Dysfunction in Parkinson's Disease: The Role of LRRK2 G2019S in Endothelial Cells

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Vascular dysfunction and blood–brain barrier (BBB) impairment are increasingly recognized as contributors to the pathophysiology of Parkinson's disease (PD). While PD is primarily characterized by dopaminergic neuronal loss and α-synuclein aggregation, growing evidence indicates that non-neuronal cells, including endothelial cells (ECs), may play an active role in disease progression. Endothelial cells are essential for maintaining BBB integrity, regulating molecular transport, and responding to vascular growth factors such as VEGF through the receptor VEGFR2. LRRK2 G2019S mutation is the most common PD causing mutation and has been shown to disrupt intracellular trafficking and kinase signaling. However, the impact of this mutation on endothelial cell biology remains poorly defined.

To address this question, induced pluripotent stem cell (iPSC)-derived endothelial cells from PD patients carrying the LRRK2 G2019S mutation and their isogenic controls were used to investigate alterations in VEGF-VEGFR2 signaling, VEGFR2 endocytosis and intracellular trafficking and expression of key endothelial markers (von Willebrand Factor (vWF), in both cell types. We also investigated the levels of α-synuclein expression.

Our findings indicate that the LRRK2 G2019S mutation leads to altered VEGFR2 endocytosis accompanied by modified VEGF signaling responses. Furthermore, changes in vWF and α-synuclein expression were observed. These results highlight a previously underappreciated role of endothelial cells in PD and suggest that vascular and BBB dysfunction, mediated through aberrant LRRK2 signaling, may contribute to disease mechanisms beyond the neuronal compartment.



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MDM2 as a precision therapeutic target in muscle-invasive bladder cancer: Insights from PDX models and clinical cohorts

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The heterogeneity of bladder cancer (BlCa) prognosis necessitates patients' lifelong surveillance, emphasizing the need for the development of modern molecular tools towards personalized therapeutics. Herein, we have explored BlCa copy-number alterations (CNAs) landscape, utilizing bladder tumors of 11 patient-derived xenograft (PDX) mouse models and 243 BlCa patients.

Targeted-DNA-seq and poly-A+ RNA-seq (MGI DNBSEQ-G400 platform) in PDX tumors revealed E3 ubiquitin-protein ligase proto-oncogene *MDM2* to be aberrantly amplified/overexpressed and strongly correlated with worse PDX survival (p=0.028). Next, focusing on *MDM2* CNA analysis in patients' tumors, we revealed that *MDM2* gain/amplification (16.8%) was significantly associated with *MDM2* overexpression, and with increased risk for post-treatment metastasis (HR=2.849, p=0.011) and poor overall survival (HR=2.083, p=0.023) of muscle-invasive BlCa (MIBC) patients. The analysis of 5 external validation cohorts (TCGA-BLCA, n=408; MSK-MetTropism-BlCa, n=961; MSK-Eur-Urol-2014; n=109, IMvigor210; n=448, Sjödahl cohort; n=149) further confirmed the inferior clinical utility of *MDM2* in terms of gain/amplification and/or overexpression.

Cisplatin-based chemotherapy and radiation are the standard-of-care treatments of MIBC patients. Prompted by the established MDM2–p53 pathway disruption in response to cisplatin/radiotherapy-induced DNA damage, we explored MDM2 biological oncogenic role in BlCa. *In vivo* PDX treatment-studies (Jackson Laboratory-MMHCdb) correlated *MDM2* amplification/overexpression with increased cisplatin resistance, while in TCGA-BLCA, patients with *MDM2* amplification/overexpression who received platinum-based chemotherapy/radiotherapy displayed significantly inferior outcomes. *In vitro* MDM2-siRNA-mediated knockdown (RT4/5637/T24 cell lines) significantly reduced cell proliferation/migration and colony formation, enhanced apoptosis levels and most importantly increased cisplatin/radiation sensitivity both in 2D and 3D cultures. Interestingly, the combination of cisplatin, radiation and MDM2-knockdown outperformed the therapeutic efficacy of each treatment alone, resulting in sufficient eradication of cell growth both in 2D and 3D conditions.

Collectively, our study identified *MDM2* amplification/overexpression as a potent molecular tool that could be used beyond clinical staging in improving MIBC patients' risk-stratification, as well as a novel therapeutic target, revolutionizing precision medicine decisions.

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P65 - 264

Circulating miRNA risk score in bladder cancer: translational impact from patient-derived xenografts to patients' progression and treatment outcome

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Bladder cancer (BlCa) monitoring demands lifelong follow-up with invasive procedures, aggravating patients' quality-of-life and leading to significant healthcare costs. In this regard, minimally-invasive precision medicine is of first clinical priority. Herein, we have explored BlCa patients' circulating miRnome, with the goal of uncovering their potential clinical utility.

miRNA-seq was performed in 15 BlCa patient-derived xenograft (PDX) mouse model tumors (n=15) and matched plasma (n=15) samples (MGI DNBSEQ-G400 platform). The significantly altered miRNAs in PDXs were quantified in 216 BlCa patients' circulation at diagnosis following 3'-end polyadenylation & RT-qPCR. Cox proportional hazards regression analysis was applied to develop a circulating miRNA-based risk-score (miRisk-score), specific for non-muscle invasive BlCa (NMIBC) and muscle invasive BlCa (MIBC), based on patient's relapse/progression and mortality, respectively.

miRNA-seq demonstrated the concurrent upregulation of miR-100, miR-1246, miR-1290, miR-193b, miR-375 and miR-4488 in both PDX tumors and plasma, strongly correlated with advanced stage/grade, and early PDX mouse models progression. Their analysis in our screening cohort strongly validated their correlation with adverse clinicopathological disease features, while elevated pre-treatment circulating miR-375/miR-4488 and miR-1290/miR-193b levels were associated with worse NMIBC and MIBC outcome, respectively. Following, Cox proportional regression analysis applied to establish an independent NMIBC-specific (miR-375/miR-4488) and MIBC-specific (miR-1290/miR-193b) miRrisk-score, which outperformed the prognostic value of each individual circulating miRNA. NMIBC patients with higher miRisk-score were associated with significantly increased likelihood of early relapse (HR=1.839; p=0.036) and progression (HR=3.342; p=0.002), while incorporating the miRisk-score into multivariate models improved the risk-stratification of Ta/T1low-grade patients. In addition, higher miRisk-score was correlated with worse survival of T2-T4 patients (HR=1.839; p=0.036), while its integration with tumor stage enhanced risk prediction.

Collectively, evaluation of these newly established NMIBC- and MIBC-specific circulating miRisk-scores could provide a minimally-invasive tool to complement biopsies, potentially reducing unnecessary invasive follow-up procedures and guiding personalized treatment in BlCa.

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The effect of lipid metabolism regulator Angiopoietin-like-3 protein (ANGPTL-3) on the rate-limiting step of cholesterol biosynthesis during Hepatitis C virus (HCV) infection

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The lipid metabolism regulator ANGPTL-3 increases plasma lipids through inhibition of lipoprotein and hepatic lipases, thereby reducing lipoprotein clearance and causing intracellular lipid shortage. HCV-orchestrated reprograming of host lipid metabolism is vital for the establishment of HCV infection. It leads to hepatic fibrosis and steatosis that could progress to hepatocellular carcinoma (HCC), even after virus eradication with direct-acting antivirals. We have previously shown that HCV down-regulates ANGPTL-3 at the early stages of infection. Elevated ANGPTL-3 reduces intracellular triglycerides and lipid droplets, both crucial to HCV propagation, and effectively inhibits viral replication. The present study aimed to investigate whether ANGPTL-3 attenuates intracellular cholesterol biosynthesis as a complementary antiviral mechanism. Thus, we examined mRNA and protein expression of the rate-limiting enzyme of cholesterol biosynthesis, 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR), in HCV permissive Huh7.5 hepatoma cells and daughter cell line G4 that overexpresses ANGPTL-3. Gene expression changes of the upstream kinase AMPK and Ser872 phosphorylation status of HMGCR were used to assess enzymatic activity. Atorvastatin-treated cells were used as a positive control for altered HMGCR expression. We observed an inversely proportional relationship between HMGCR mRNA and protein expression with time in both Huh7.5 and G4 cells. ANGPTL-3 overexpression caused a dramatic decrease in HMGCR protein during a four-day cell culture, mimicking atorvastatin treatment for 48 hours. This reduction was accompanied by increased HMGCR phosphorylation and enhanced AMPK mRNA levels, suggesting inactivation of the enzyme. HCV infection of G4 cells managed to restore HMGCR expression and greatly reduced protein phosphorylation compared to mock-infected controls. Taken together, our results demonstrated that ANGPTL-3 differentially modulated the rate-limiting step in cholesterol biosynthesis in HCC. Despite this, HCV infection could overcome the ANGPTL-3-driven effects to keep propagating.

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P67 - 709

Elastin-derived peptides (EDPs) modulate breast cancer cell behavior in 2D and 3D cell models

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The development and progression of cancer are tightly influenced by the signals received from the extracellular matrix (ECM). ECM is a highly dynamic 3-dimensional network composed of several macromolecules that not only provide structural support but also regulate cell behavior. Among its components, elastin is a long-lived protein responsible for the structural integrity and elasticity of tissues. Although it has high stability, elastin undergoes progressive fragmentation, a process known as elastosis, which results in both the abnormal deposition of elastotic masses and the release of elastin-derived peptides (EDPs), known as elastin matrikines. Unlike intact elastin, EDPs can act as bioactive signals that can alter cell behavior and have been implicated in cancer progression by enhancing the invasiveness of breast cancer cell lines.

This study aims to investigate the impact of matrikines on two breast cancer cell lines, MDA-MB-231 and shERβ MDA-MB-231 and to evaluate the role of estrogen receptor β (ERβ) in mediating these responses. We focus on key functional properties such as migration, dissemination, growth, and the expression of ECM molecules utilizing both two-dimensional (2D) and three-dimensional (3D) culture models. Our findings reveal that EDPs enhance the invasive potential of the cells.

Notably, the expression levels of elastin-related molecules, including cathepsin A and elastin-binding protein, differ depending on the culture dimensionality. These results highlight the significance of elastin fragmentation and ERβ expression in modulating the tumor microenvironment and influencing breast cancer aggressiveness and support the idea that 3D cell platforms mimic the tumor microenvironment *in vivo* decoding the involvement of EDPs in cancer progression.



P68 - 16

Epigenetics: Advancing Biomarker Discovery for Prostate Cancer Disease States

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Background: Prostate cancer (PCa) remains a challenging malignancy, primarily due to the absence of reliable biomarkers that can accurately determine disease state and thereby inform optimal patient management strategies. We suggest that altered CpG sites may serve as promising biomarkers for the identification and monitoring of distinct disease states throughout the clinical course of PCa patients. Ultimately, this research aims to facilitate marker-informed anticipatory interventions and candidate therapeutic targets to combat prostate cancer.

Methods: In total, sixty-four formalin-fixed paraffin-embedded (FFPE) samples were collected from PCa patients encompassing specimens from normal prostate, and indolent, high grade and aggressive PCa. Tissue slides were reviewed, and approximately fourteen slides were macrodissected to increase tumor content for each case. Samples underwent DNA extraction, followed by DNAme analysis using the Infinium Methylation EPIC v2.0 array. In addition, 370 public datasets were queried and retrieved from TCGA-PRAD and GEO databases representing the whole spectrum of PCa to further validate our experimental results. For all data, beta-value transformation of .idat files was performed using the minfi package, and beta values in the range of 0.31–0.59 were excluded prior to standard differential methylation analysis, which was performed using the limma package. A cutoff of Delta-beta≥|±0.4| was used to identify significantly hypermethylated/hypomethylated CpG sites. Machine learning was also employed using scikit learn package and further enrichment analysis was performed using Enrichr platform.

Results: Ongoing analyses explore candidate epigenetic signatures that will stratify patients' tumors based on disease state and progression status. Up to date, an epigenetic signature of 680 epigenetically altered genes has been able to distinguish normal to cancer samples and determine distinct disease states. Our overall goal is to provide an epigenetic signature of less than 100 epigenetically-related markers that will help performing best practices in the clinic for patients with prostate cancer.



P69 - 17

Lysine Methyltransferase SUV4-20H2 activity contributes to the mesenchymal phenotype and migration potential of Pediatric Astrocytomas

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Objective: Post-translational modifications of histones represent a major mechanism of epigenetic regulation of gene expression, influencing chromatin structure and the binding sites of key transcription factors and regulators. Histone post-translational modifications participate in critical cellular processes, including DNA replication and repair, gene expression, chromatin compaction, and cell cycle control. Early analysis of the epigenetic profile of pediatric astrocytomas revealed high levels of histone H4 trimethylation (H4K20me3) in neoplastic cells compared to normal brain tissue, suggesting a potential role of the lysine methyltransferase SUV4-20H2 in tumor development. The aim of this study was to investigate the functional role of SUV4-20H2, which mediates H4K20me3 modification, in pediatric astrocytomas.

Materials and Methods: The protein expression of SUV4-20H2 and H4K20me3 was assessed by immunohistochemistry in 46 pediatric astrocytoma tissue samples and 5 archival normal brain tissue samples. Expression levels were correlated with clinicopathological features and patient survival. The functional significance of SUV4-20H2 in tumor cell proliferation, migration and apoptosis was evaluated using the selective inhibitor A-196 in pediatric astrocytoma cell lines (SJ-GBM2, CHLA-200) in vitro. A-196 effects in epithelial mesenchymal transition (EMT) markers and apoptosis were evaluated by western immunoblotting.

Results: Significantly higher levels of H4K20me3 and SUV4-20H2 were observed in pediatric astrocytomas as well as in high-grade pediatric astrocytoma cell lines (SJ-GBM2, CHLA-200) compared to normal tissues. Pharmacological inhibition of SUV4-20H2 by A-196 resulted in a marked reduction of cell viability and migration of pediatric astrocytoma cells at 48 and 72 hours in vitro, followed by a significant reduction in EMT markers (N-cadherin, vimentin, β-catenin) and increased PARP cleavage.

Conclusions: Our findings demonstrate a potential epigenetic oncogenic role of SUV4-20H2-dependent H4K20me3 in pediatric astrocytomas, contributing to their mesenchymal/invasive characteristics, and its pharmacologic inhibition represents a promising therapeutic strategy to suppress tumor progression.



P70 - 75

The role of histone demethylase PHF8 in macrophage type I interferon response and mitochondrial function

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Macrophages are phagocytic cells of the innate immunity, that constitute the first line of defense against infections. Macrophages mediate inflammation in response to diverse environmental cues. Their activation manifests through a spectrum of phenotypic states, ranging from classical pro-inflammatory "M1-like" to alternative anti-inflammatory "M2-like" and their intermediates. Macrophage polarization requires tight transcriptional regulation of signaling cascades. Epigenetic modifications also play a vital role in coordinating macrophage plasticity and metabolism, although their mechanism of function remains to be elucidated. PHF8 (KDM7B) is a histone lysine demethylase, involved in neurodevelopment, cancer progression and inflammation. During acute TLR4-driven inflammation, PHF8 promotes T cell activation via macrophage secretome modulation. PHF8 has been proposed to be a molecular safeguard of IFN-γ response, whereas it modulates metabolism through mTOR hyperactivation in the hippocampus of PHF8 knockout mice.

To this point, we aim to unravel the potential effect of PHF8 on type I interferon signaling and its role in mitochondrial homeostasis in macrophages. To do so, we created CRISPR-Cas9 engineered stable macrophage cell lines (RAW264.7) with PHF8 knock-out and PHF8 overexpression.

In this work, we quantified the expression levels of interferon-stimulated genes (ISGs) upon macrophage activation, showing that PHF8 differentially regulates these genes in naïve, unstimulated cells and in cells activated by LPS. Since ISG expression can be tightly associated with mitochondrial homeostasis, we investigated the role of PHF8 in mitochondrial function. We showed that PHF8 compromised mitochondrial function with reduced mitochondrial biomass, lower membrane potential, decreased ATP production and basal respiration rate in macrophages overexpressing PHF8. Overall, our results, suggest that PHF8 is a promising regulator of macrophage metabolism and immune responsiveness of macrophages, bridging epigenetics with immunometabolism.



P71 - 88

GemC1 modulation drives genome-wide chromatin changes shaping the fate decisions between NSCs and ependymal cells

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In the developing telencephalon, radial glial cells (RGCs) give rise to neural stem cells (NSCs) and ependymal cells (ECs). A population of RGCs differentiate into quiescent NSCs (qNSCs) in the subventricular zone, remaining dormant and capable to reactivate and generate neurons and glia throughout adulthood. Another subset of radial glial cells commits to the ependymal cell lineage during late embryonic development. Ependymal cells are ciliated epithelial cells that line the ventricles of the brain, playing a vital role in cerebrospinal fluid circulation and brain homeostasis. We have previously provided evidence that two proteins of the Geminin superfamily, GemC1 and McIdas, govern the ependymal cell fate molecular pathway. Prior research from our laboratory established that in the absence of GemC1, radial glial cells are directed toward NSC fate acquisition, while GemC1 overexpression reprograms neural stem cells toward the ependymal lineage. To uncover the underlying mechanism governing GemC1-mediated fate decisions in neural stem cells, we performed ATAC-seq analysis on the chromatin landscape of GemC1 knockout radial glial cells. Our results showed that ependymal-associated genes exhibited a closed chromatin state, whereas NSC-related genes displayed roughly twofold more accessible promoters. These data indicate that GemC1 orchestrates radial glial cell fate commitment by restricting chromatin accessibility at NSC-specific promoters. Furthermore, our results showed that GemC1 loss alters Polycomb (H3K27me3) targeting and SWI/SNF (Brg1) recruitment at ependymal and neuronal gene promoters respectively. Our data support the idea that fate decisions between NSCs and ependymal cells are closely interconnected and further investigation on the role of GemC1 will provide new insights into the understanding of the fate decisions of post-natal neural progenitor cells in the mammal brain.

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Effects of a Greek Yoghurt–based Complex on the Human Facial Skin Microbiome

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Background/Aim: A series of natural ingredient-containing complexes are being used in dermo-cosmetics to ameliorate facial characteristics, but human experimental trials demonstrating their efficacy remain limited. The purpose of this study was to assess the effects of a greek yoghurt–based complex on the human facial skin microbiome and its ability to induce re-balancing effects on a human face ecosystem that exhibits dysbiosis.

Materials and Methods: Thirty-one volunteers (26 females, 5 males; aged 20-65 years) with signs of stressed facial skin participated in a 28-day observation of the effects on the skin microbiome of a yoghurt-based formulation. Skin swab samples were collected non-invasively in four time points post-application of the formula (t_0=0 days, t_1=7 days, t_2=14 days, t_3=28 days). The results were compared to a volunteer group with healthy balanced skin along with the "placebo" group. Bacterial DNA was extracted and analyzed using 16S rRNA gene amplicon sequencing to characterize the microbial communities present. The data was then processed using bioinformatics techniques and the taxonomic composition, diversity and relative abundance of the microbiome were assessed.

Results: A lowered microbiota diversity was observed in "stressed" facial skin, compared to the "balanced" group. The four most abundant microbiota genera observed included *Corynebacterium*, *Propionibacterium*, *Staphylococcus* and *Actinomyces* spp., in both groups. However, there was a significant difference in the relative abundance of these species in the two groups (stressed-balanced). Intervention with the yoghurt-based complex was found to be associated with a consistent increase in mean bacterial diversity, gradually restoring the facial skin's microbiome balance over time.

Conclusion: The yogurt-based complex potently promoted the restoration of the microbiota's physiochemical balance by reducing the overrepresentation of certain bacterial species, while simultaneously enhancing relative abundance of others in an "imbalanced" skin. This counteracts the microbial imbalance present in stressed skin, promoting stabilization of the facial skin ecosystem and the recovery of homeostatic mechanisms essential for a healthy skin function and appearance.



P73 - 113

Construction and Characterization of Prostate Cancer Tissue Microarrays: A High-Throughput Tool for Molecular Profiling of Disease Progression

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Prostate cancer (PCa) describes a highly heterogenous cancer. Immunohistochemistry (IHC) remains a fundamental technique for assessing protein expression, offering critical insights into disease mechanisms and biomarker discovery. Conventional IHC analysis is often constrained by inter-slide variability, high reagent consumption, and the time-intensive process of staining and evaluating large sample cohorts individually. Tissue microarrays (TMAs) enable high-throughput, standardized analysis of numerous samples, thereby advancing biomarker identification, validation, and translational cancer research. Preliminary studies showed altered methylation of PHB, KRT15 and GDF15 in patient-derived xenograft models of aggressive PCa compared to benign tissue. We developed patient-derived prostate cancer (PCa) TMAs representing a broad disease spectrum to evaluate protein expression of the candidate epigenetically-regulated biomarkers.

Three TMAs encompassing specimens from 63 PCa patients were constructed, including 32 normal prostate tissues, 37 low-grade tumors (Gleason Score 6–7), and 22 high-grade tumors (Gleason Score 8–10). Haematoxylin and eosin–stained slides from all specimens were reviewed, and representative areas of the dominant tumor and adjacent benign tissue were selected for inclusion. In mixed tumors, both adenocarcinoma and neuroendocrine components were included. Each case was represented by a median of five tissue cores measuring 0.6–1 mm in diameter. This diverse cohort captures key clinical and pathological stages of prostate cancer progression.

TMAs were used to conduct IHC analyses for GDF15, KRT15, and PHB. Ongoing analyses show altered protein levels and provide valuable insights into the epigenetic and molecular pathways involved in prostate cancer progression and therapeutic resistance. This approach highlights the potential of integrating methylation profiling with tissue-based protein expression studies to identify clinically relevant biomarkers and novel therapeutic targets.

Overall, TMA establishment enables systematic evaluation of biomarkers and serves as a powerful tool for the identification and validation of novel prognostic and therapeutic markers.



P74 – 134

Investigation of the effect of HIV-1 insertion site on the transcriptional regulation of the virus and the interleukin-2 (IL-2) gene via chromosomal interactions

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We and others have confirmed significant homology between the sequences of the 5′ long terminal repeat (LTR) region of HIV-1 and the IL-2 promoter, resulting in their common transcriptional regulation in infected CD4+ T helper (Th) cells. The transcription factor Ets-2 acts as a transcriptional repressor of both IL-2 and HIV-1 in naive Th cells, while in activated cells, Ets-2 mRNA and protein levels are reduced, allowing the expression of IL-2 and HIV-1. Using J-Lat 6.3 cells (derived from the Jurkat T cell line and containing a latent, integrated HIV-1 provirus), we detected a trans-chromosomal interaction between the IL-2 promoter and the HIV-1 LTR, which disappears after cell activation. Because the virus can integrate into different sites in the genome, with a preference for transcriptionally active gene loci and super-enhancer structures, we investigated whether differential viral integration affects the IL-2/HIV-1 interaction and subsequently the expression of IL-2, Ets-2 and the virus. We used J-Lat 6.3 and J-Lat 8.4 cells, both latently infected with a non-infectious HIV-1 provirus integrated into different chromosomal sites. As controls, we used uninfected Jurkat T cells, J-LTRG T cells (containing only the HIV-1 LTR), and A2 T cells (containing only the HIV-1 Tat gene). In all cells carrying HIV-1 elements, the LTR was linked to a GFP reporter gene. Total RNA was isolated from unstimulated and 6-hour-stimulated cells, and qPCR analysis was performed to evaluate the expression levels of IL-2, Ets-2, Tat and GFP. Our results showed that the insertion site *per se* did not influence the expression pattern of the above genes. Furthermore, our preliminary results from chromosome conformation capture (3C) experiments suggested that the insertion site does not affect the interaction between the IL-2 promoter and the HIV-1 LTR. Our ongoing work aims to delineate the exact molecular mechanism of IL-2 and HIV-1 co-regulation.



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Therapeutic Efficacy and Molecular Impact of the Epigenetic Modulator Panobinostat in Aggressive Prostate Cancer Patient-Derived Xenografts

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Prostate cancer (PCa), the second leading cause of cancer-related mortality in men, is characterized by significant molecular and clinical heterogeneity. We and others have hypothesized that epigenetic alterations, including DNA methylation and histone acetylation, play a pivotal role in tumor initiation, progression, and therapeutic resistance at the disease course of PCa. Aggressive variants of prostate cancer (AVPC) represent an androgen receptor (AR)-independent disease state with distinct morphological, genetic, and epigenetic characteristics. The MDA PCa 144-4 (AR-negative large-cell neuroendocrine carcinoma) and MDA PCa 177-B (AR-negative adenocarcinoma with a basal phenotype) are unique Patient-Derived Xenograft (PDX) models originally established at MD Anderson Cancer Center and currently established at the University of Patras. Preliminary analyses revealed distinct DNA methylation profiles compared to non-malignant tissues, supporting the notion that epigenetic dysregulation contributes to the aggressiveness of AVPCs. Based on this rationale, we explored the effect of the histone deacetylase inhibitor Panobinostat as a potential epigenetic therapeutic strategy. To mimic the human disease, C.B-17 SCID male mice (~5 weeks) underwent patient-derived tumor implantation followed by intraperitoneal drug administration in tumor volume equal or more than 250mm^3. Panobinostat was tested in both MDA PCa 144-4 and 177-B models. A control group (vehicle/placebo) for each model was also included. Following treatment (10mg /kg, three times per week for two weeks of administration), tumor tissues were harvested for morphological and molecular (i.e. immunohistochemistry for key epigenetic biomarkers) analyses. Treatment with Panobinostat resulted in significant slower tumor growth compared to controls, without detectable alterations in cell morphology or expression of standard markers (PSA, synaptophysin). Molecular analyses of epigenetic biomarkers are ongoing to elucidate the mechanistic basis of these effects. Future studies will test Panobinostat alone and in combination, aiming to identify predictive epigenetic biomarkers to guide personalized therapies and lead to best practices for patients with prostate cancer.



P76 - 209

HIV-Driven Transcriptional Modulation of Human Endogenous Retroviruses in immortalized T-lymphocyte cells

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Human endogenous retroviruses (HERVs) are remnants of ancient viral infections that comprise approximately 8% of the human genome. While most HERV loci are transcriptionally silent in healthy tissues, their expression can be modulated in cases of proliferative disorders and neurodegenerative and autoimmune diseases as well as under conditions of viral infection. HIV infection, in particular, has been reported to alter host transcriptional programs, yet studies regarding its impact on HERV activity have produced contradictory results. Our goal is to clarify the effect of HIV-HERV interplay by investigating the transcriptional rewiring of HERV-bearing and HERV-flanking genes. We infected Jurkat cells with HIV-1 from patients' plasma and used the extracted RNA from the infected as well as uninfected (control) Jurkat cultures for high-throughput whole transcriptome sequencing (RNA-seq) experiments. We proceeded to the bioinformatic analysis of the RNA-seq datasets produced, to resolve alterations in HERV expression levels following infection with HIV. We employed a family-wide approach for the characterization of the expression of HK2 universally, as we have previously proven that per locus resolution is particularly challenging and error-prone during the expression study of LTR-transposable elements. Our analysis revealed an upregulation of HERV transcripts in nearly half of the families tested, indicating a broad transcriptional activation across distinct HERV groups. These findings demonstrate that HIV infection can reshape the transcriptional landscape of HERVs in immortalized T-cell lines. Such reactivation may contribute to immune recognition, influence viral replication dynamics, or modulate host gene expression through HERV-derived regulatory sequences.



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Investigating syncytial expression in placenta during COVID-19 infection

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Human Endogenous Retroviruses (HERVs) are the result of ancient endogenization events, found dispersed in the human genome. Most HERVs have been silenced over evolutionary time through epigenetic regulation and mutations. A notable exception is syncytin-1, encoded by the *env* gene of a HERV-W family provirus, which is essential for placental formation. Immunopathogenic conditions, including multiple sclerosis and preeclampsia, have been previously linked to aberrant syncytin-1 expression. In our study, we aimed to test whether syncytin-1 production is altered in placentas of COVID-19 infected mothers, motivated by emerging evidence of HERV activation in infected patients. Publicly available RNA-Seq placenta samples of infected mothers (n=17) and healthy (n=4) as well as positive (placentas with other pathologies) controls (n=4) were analysed. Our bioinformatics protocol included reads quality control, filtering, and splice-aware alignment to the human reference genome. HERV expression was quantified by aggregating the reads from each HERV family, and normalizing counts based on the expression of tissue-specific housekeeping genes. Independent samples t-tests were performed to assess differential expression. As a complementary analysis, long read healthy placenta RNA-Seq data were incorporated to get an overview of the relative isoforms expressed in the tissue using both sequencing technologies in normal conditions. Preliminary results indicate significant downregulation of HERV-K (HML-1) and HERV-FRD ($p<0.05$) in COVID-19 patients compared to healthy and positive controls, respectively. Comparison of healthy and positive controls revealed significant downregulation of both HML-1 and HERV-9 ($p<0.05$), while HERV-FRD appeared to be preferentially activated in non-COVID immunopathogenic contexts ($p=0.03$, $p=0.033$ compared to COVID-19 and negative control group, respectively). No significant differential expression of syncytin-1 was observed among the tested groups. However, we found certain isoforms showing consistently higher abundance within samples without association to infection. Further studies are needed to explore any potential functional implications of syncytin-1 and its isoforms in COVID-19 response.



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A screening approach for uncovering functional insulators to improve safety and durability of gene therapy vectors.

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Gene therapy employing genome-integrating viral vectors has achieved major progress in addressing previously untreatable genetic disorders. Nonetheless, significant obstacles persist, particularly transgene silencing and insertional mutagenesis arising from random genomic integration. Chromatin insulators—regulatory DNA sequences that establish transcriptional boundaries—represent a promising strategy to mitigate these risks. These elements possess two core functions: enhancer-blocking, which prevents inappropriate enhancer–promoter communication, and barrier activity, which shields transgenes from heterochromatin-induced repression. Incorporating insulators into viral vectors may therefore enhance transgene expression stability while minimizing genotoxic outcomes, a concern underscored by hematologic malignancies observed in hematopoietic stem cell (HSC) gene therapy trials. However, current assays to evaluate insulator activity are inadequate, typically relying on episomal reporter systems lacking chromatin context or on labor-intensive *in vivo* models. To address these limitations, we established an integrated, locus-defined platform enabling concurrent assessment of enhancer-blocking and barrier functions. Using K562 cells, a GFP reporter cassette driven by the weak HBG-201 promoter and regulated by either a strong (SKA1) or moderate (HS3) enhancer was precisely inserted into the AAVS1 safe-harbor locus. Candidate insulator sequences were positioned between enhancer and promoter. Effective enhancer-blocking was inferred from decreased GFP expression, indicating disruption of enhancer–promoter communication. Barrier function was evaluated via dCas9-KRAB–mediated repression of the upstream PPP1R12C gene: in the absence of insulation, repressive chromatin spread to silence the reporter, whereas functional barriers impeded propagation. Robust enhancer-blocking was observed for insulator C1 in reverse orientation and for the composite A2C1 construct. Substitution of SKA1 with HS3 enhanced activation of insulator effects, suggesting that excessive enhancer strength can obscure insulation (11.1% increase in relative activity). Interestingly, the potent SKA1 enhancer resisted CRISPRi-mediated heterochromatin spreading, while HS3 or enhancer omission permitted silencing. This system provides a powerful and context-relevant framework for insulator validation, facilitating safer and durable gene therapy outcomes.



P79 - 141

A solid phase method for detection of Src protein tyrosine kinases activity

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Src protein tyrosine kinases are enzymes heavily implicated in the intracellular signalling cascades. They are involved in the regulation of metabolism, viability, proliferation, differentiation and migration of many different cells. The Src family consists of eight members, namely Lyn, Hck, Lck, Blk, c-Src, Fyn, Yes and Fgr, which can be grouped into two subfamilies, those that are Lyn related (Lyn, Hck, Lck and Blk) and those that are c-Src related (c-Src, Yes, Fyn and Fgr), showing similar modes of regulation and activation. While these enzymes have important normal cellular activities, many members have also been implicated, either directly or indirectly, as being hyperactive, in various diseases including cancer, making them the largest group targeted for the development of therapeutic inhibitors.
In this study, a solid phase assay was developed in order to detect the activity of Src kinases, without the use of radioactive ATP and autoradiography. Biotinylated peptides were synthesized, containing the tyrosine in an appropriate sequence, and used as substrates for Src kinases. The peptides were synthesized in solid phase on 2-chlorotritylchloride resin (CLTR-Cl), according to Fmoc/tBu strategy, using *N,N*'-diisopropylcarbodiimide (DIC) and 1-hydroxybenzotriazole (HOBt) as coupling reagents. The purification of peptides was performed by semi-preparative high-performance reverse-phase liquid chromatography (semi-Preperative HPLC) and was checked by analytical high-performance reverse-phase liquid chromatography (Analytical HPLC). Their identification was achieved by mass spectrometry (ESI-MS). For solid phase assays, standard active Lyn kinase and 96-well polystyrene plates coated with streptavidin were used. A specific antibody for phosphotyrosine, peroxidase conjugated (anti-P-Tyr-HRP), was used to detect phosphorylated tyrosines. The methodology developed is simple, without special manipulations, with a very small coefficient of variation (CV), (Intra assay, CV: 4.16%-8.96%), and (Inter assay, CV: 5.99%-11.15%), showing high accuracy and repeatability.



P80 - 310

Live Imaging and Machine Learning Reveal Context-Dependent Roles of Su(H) in Embryonic Transcriptional Bursting

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How broadly expressed transcription factors (TFs) achieve spatially precise regulation of gene expression, during early embryonic development remains a central question in developmental biology. Transcriptional bursting offers a powerful new framework to explore gene regulation on a new level at spatial and temporal resolution. Building on our previous findings, that identified Suppressor of Hairless [Su(H)] as a repressor at the dorsal borders of the *short gastrulation (sog)* expression domain, we applied super-resolution MS2-MCP live imaging to directly visualize nascent transcripts of the TGF-β/BMP antagonist *sog* in *Drosophila melanogaster*. This approach enabled single-cell quantification of transcriptional bursting dynamics in real time. Our data demonstrated that Su(H) exhibits dual regulatory roles—acting as a repressor at the dorsal border while stabilizing transcriptional bursts in ventral regions of the expression domain simultaneously. Loss of Su(H) binding led to more frequent but weaker bursts and delayed transcriptional onset, revealing a context- and stage-dependent regulatory mechanism.

Building on these findings, implemented a machine learning pipeline (XGBoost, Ripley's K-function) to predict the spatiotemporal dynamics of active nuclei across developmental stages. The model accurately reproduced enhancer-driven expression patterns in both wild-type and Su(H)-mutant embryos and captured Su(H)'s context-dependent behavior. This computational framework enables *in silico* enhancer mutagenesis, allowing prediction of how specific motif alterations affect transcriptional activity and spatial expression.

Tracking the cellular localization, expression patterns, and interactions of *sog and Su(H) acts as an example to* offer deeper insight into molecular mechanisms driving brain development. This approach also facilitates identification of key events such as lineage specification and differentiation in a spatiotemporal context. Together, these approaches bridge molecular and computational perspectives to reveal how a single transcription factor integrates positional information to ensure robust and precise gene expression during early embryonic patterning.

Keywords: Su(H), *Drosophila*, transcriptional bursting, enhancer regulation, gene expression dynamics, live imaging, machine learning



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Generation of a KLK5-specific activity-based probe (KLK5-ABP) for detection of the active KLK5 protease in biological and clinical samples

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KLK5 is a trypsin-like serine protease expressed in the stratum granulosum, the most external living layer of the epidermis, where it has a dominant role in regulation of physiological skin desquamation. Elevated KLK5 activity has been causally linked to pathology in inflammatory skin diseases, like Netherton syndrome, atopic dermatitis, and rosacea [1]. Consequently, tools to monitor the active form of KLK5 in normal and disease states are valuable over antibodies that determine the total amount of the enzyme (that may be in an inactive state, *i.e.,* zymogen, cleaved or complexed with inhibitors). In this respect, activity-based probes (ABPs) are chemical entities used to label and quantify or localize the active form of an enzyme. ABPs are composed of three distinct parts: the reactive group or warhead that binds to the active site, the recognition sequence (determinant of specificity), and the tag (a fluorophore or biotin), which enables detection. ABPs can also act as target-specific suicide inhibitors, therefore, they can be developed to potential therapeutics (theranostics) [2]. To develop a KLK5-specific ABP, a previously identified KLK5-specific tetrapeptide substrate [3] was modified: (a) the P1 Arg residue was replaced by the O,O-diphenyl phosphonate analog of Arg to act as a warhead, and (b) the fluorescent dye Texas Red was incorporated at the *N*-terminus, as tag. The KLK5-ABP was chemically synthesized, purified by HPLC and verified by mass spectrometry. The specificity of the KLK5-ABP for KLK5 over closely related proteases and the limit of detection of KLK5 were determined with Western blot using active recombinant proteases. Further, the ability of the KLK5-ABP to detect active KLK5 in tissues was tested by activography, an ABP-based histochemical method [4]. The potential of the KLK5-ABP to be exploited as a drug was validated using three-dimensional organotypic human skin substitutes, specifically, Netherton syndrome skin and normal skin.

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IN3-Structure: AI-Enhanced Ligand-Receptor Profiling for Drug Repurposing

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G protein–coupled receptors (GPCRs) form the largest and most pharmacologically relevant family of membrane receptors, mediating the transduction of diverse extracellular signals (including photons, lipids, neurotransmitters, and hormones) into precise intracellular responses. Despite their conserved seven-transmembrane (7TM) topology, GPCRs are conformationally dynamic entities that exist as ensembles of functionally distinct states[1]. Activation involves characteristic structural rearrangements, such as the outward displacement of transmembrane helix 6 (TM6) and reorganization of key intracellular microswitches, which govern the engagement of downstream effectors and signaling outcomes[2].

Despite over 300 high-resolution GPCR structures being experimentally resolved, these snapshots capture only a fraction of the conformational landscape[3]. This limitation obscures the molecular determinants of state-dependent ligand binding, signaling bias, and allosteric modulation, which are critical parameters for rational drug design and repurposing. Given that ~40% of FDA-approved drugs target GPCRs and ~92% are small molecules[4], exploiting this structural complexity could unlock new therapeutic opportunities.

We present a computational pipeline that integrates AlphaFold3 structure prediction, Boltz-2 affinity modelling, and GNINA pose scoring to generate high-resolution models of Family A GPCRs bound to agonists and antagonists in active and inactive states. By modelling ligand-bound conformations across functional states, we capture structural determinants that conventional docking approaches overlook. Scaling this framework to all FDA-approved small molecules enables systematic exploration of receptor-ligand space and identification of repurposing candidates.

This integrative approach bridges structural prediction with pharmacological profiling, providing a scalable strategy for mapping ligand specificity, prioritizing high-confidence interactions, and guiding experimental validation. Ultimately, it lays the groundwork for accelerated GPCR-targeted drug discovery and the rational repurposing of existing therapeutics.

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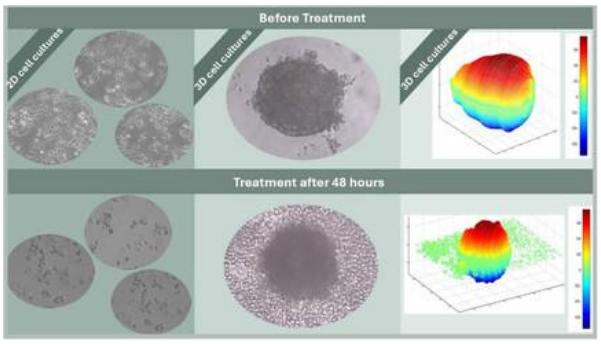
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P83 - 34

Silver(I) Metallodrugs Induce Apoptosis in 2D and 3D Breast Tumor Models: Exploring DNA and Enzyme Interactions

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The non steroidal anti-inflammatory drugs (NSAIDs) are known to accumulate in the mitochondria where the inflammation mechanism occurs. Notably, mefenamic acid (MefH), a member of the family of the fenamates modifies the permeability of the inner mitochondrial membrane, leading cells to death either by apoptosis. Moreover, MefH was found to promote cytostatic activity against various cancer types [1].
Recognizing that the metallodrugs can affect the breast cancer, the metallodrugs of formula $[Ag(tpE)_n(Mef)]$ (E= P, As, Sb; n= 2 or 3), inhibit effectively 2D monolayer cells, such as MCF-7 (hormone-dependent (HD)) and MDA MB-231 (hormone-independent (HI) cell lines. In the course of our studies on metallotherapeutics, it is suggested that the cell culture format (2D monolayer vs. 3D spheroid) plays a critical role in drug response and provides new insights into the mechanisms underlying silver(I) metallodrugs on breast cancer cells. The 3D spheroid model was studied, representing human breast cancer, was designed and applied as a clear proof of the remarkable therapeutic potential of silver(I) metallodrugs.



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P84 - 35

Targeting Microbial Resistance: Copper(I) Complexes with Triphenylphosphine and 2-Mercaptopyrimidine

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Infections caused by drug-resistant microorganisms (antimicrobial resistance, AMR) are projected to become the leading cause of mortality worldwide by 2050, with an estimated 10 million deaths annually [1]. Confronting AMR through the development of novel antimicrobial agents remains a top priority of the World Health Organization (WHO). In 2019 alone, AMR-associated infections, including those due to Escherichia coli (E. coli) and Pseudomonas aeruginosa (P. aeruginosa), accounted for nearly 4 million deaths globally [1].

Copper is well recognized for its antimicrobial properties and has been extensively employed in diverse applications, including metallodrugs, drinking water purification, and hospital hygiene [2]. 2-mercaptopyrimidine, being a pyrimidine derivative, functions as an antimetabolite and therefore plays a crucial role in interactions with nucleic acids [3]. Triphenylphosphine is frequently utilized as an ancillary ligand in the design of potential prodrugs or active drugs, as it does not substantially increase toxicity [4]. Moreover, it acts as a mitochondriotropic agent when incorporated into novel complexes, thereby enhancing their anticancer activity [5].

Our research focused on developing new metalloantibiotics that microbes have yet to resist, we synthesized and characterized copper (I) halogenated complexes with triphenylphosphine and 2-mercaptopyrimidine (CPTX, where X=Cl or Br). We employed various techniques, including FT-IR spectroscopy, XRF (solid-state study) and UV-Vis spectroscopy to fully characterize the compound. Additionally, we assessed their antimicrobial activity against *E. coli*, *P. aeruginosa, S. epidermidis,* and *S. aureus.*

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Design, Structural Features, and Biological Properties of a Triphenylantimony(V) Complex with Acetate Ligands

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Antimony compounds have long been recognized for their biological and medicinal potential, particularly in the treatment of parasitic and cancer-related diseases [1]. In recent decades, research on antimony-based therapeutics has expanded beyond traditional leishmaniasis treatments toward their use as antitumor and antimicrobial agents [2,3]. The biological behavior of these compounds depends on the oxidation state, ligand environment, and overall stability in physiological media [1,2]. Among them, organoantimony derivatives exhibit versatile coordination chemistry, enabling interactions with biomolecules, such as DNA and proteins, that can influence cellular processes including enzyme inhibition and apoptosis induction [3]. The balance between redox activity, lipophilicity, and solubility makes antimony-containing complexes attractive systems for designing novel metallodrugs [1]. Antimony(V) carboxylate derivatives, in particular, have gained attention due to their enhanced stability, lower cytotoxicity and ability to act as prodrugs that are intracellularly reduced to Sb(III) [3]. Their pharmacological performance depends strongly on the geometry and electronic properties of the metal center, which are influenced by oxygen-donor ligands [3]. Previous studies have shown that organoantimony(V) complexes bearing carboxylate or hydroxyaromatic ligands exhibit selective cytotoxicity against breast cancer cell lines and significant DNA-binding affinity [3]. These characteristics highlight the therapeutic potential of Sb(V) compounds as low-toxicity metallotherapeutic agents, capable of modulating key biochemical pathways in cancer development [2–3].

In this study, a novel triphenylantimony(V) complex with acetate ligands was synthesized by reacting triphenylantimony (Ph_3Sb) with acetic acid The compound was characterized by FT-IR, UV–Vis and ^{1}H-NMR. The complex remained structurally intact in DMSO solution. The DNA-binding affinity of the complex will be examined and the results will be comapred with the anti-proliferative activity against human breast cancer cells.

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Targeting Thyroid Cancer with a Novel Iodine-Based Complex: A Promising Therapeutic Approach

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Thyroid cancer represents the most prevalent malignancy of the endocrine system, with a steadily increasing global incidence, particularly among women [1]. The majority of cases, are classified as differentiated thyroid cancers (DTC), encompassing papillary (PTC), follicular (FTC) και Hürthle cell variants [1]. Other distinct histological subtypes include medullary thyroid carcinoma (MTC) and the aggressive anaplastic form (ATC), which is associated with poor prognosis [2]. Despite the implementation of various therapeutic modalities, such as surgical excision, radioactive iodine (RAI) ablation, external beam radiotherapy, chemotherapy, immunotherapy, and targeted agents including kinase inhibitors, the prognosis for patients with advanced or RAI-refractory thyroid cancer remains limited [3]. This underscores the ongoing need for the development of innovative treatment strategies and the integration of novel technologies into clinical practice.

During our studies on the developments of new anti-proliferate agents we report here the synthesis of a new formulation through a reaction of thioamide with iodides. The compound were characterized by FT-IR, XRF and UV-Vis spectroscopic techniques. The ligand, together with the compound, will be subjected to *in vitro* biological evaluation against the human thyroid carcinoma cell line 8505C. Furthermore, additional investigations will be conducted to evaluate the potential antibacterial properties of the synthetic compounds, thus broadening their potential biomedical applications.

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Utilization of Metabolic Data of Metal Ions in Tissues for Supporting Clinical Decision-Making.

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The diagnosis and prognosis of brain tumors represent one of the greatest challenges in modern oncology and neurosurgery [1]. Current diagnostic methods, such as Magnetic Resonance Imaging (MRI) and biopsy, while indispensable, have significant limitations. MRI provides detailed anatomical information but often fails to reliably distinguish between benign and malignant tumors or confirm metastatic nature. Biopsy, although considered the diagnostic "gold standard," is invasive, carries risks, and may not always be feasible for tumors located in sensitive brain regions. Moreover, histopathological analysis can take days to weeks, delaying treatment decisions.

In the course of our studies on the field of Metallomics [1] we are developing an innovative decision-support algorithm capable of classifying brain tumor types based on the analysis of metallic trace element concentrations (e.g., Fe, Zn, Cu, Ca, K, Cl) in tissue biomarkers, obtained through advanced analytical techniques such as X-ray Fluorescence (XRF). These elemental profiles reflect the metabolic and molecular characteristics of tumors, offering a unique "fingerprint" for diagnosis. By integrating such data with machine learning approaches, the project seeks to enable early, minimally invasive or non-invasive diagnosis, reliable discrimination between benign and malignant forms, tumor grading and classification, and ultimately, improved therapeutic decision-making.



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P88 - 49

Investigating the Biocompatibility of Advanced Materials for Neural Interfaces under the IN3-Bio Framework

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NerveRepack is an innovative project aimed at developing next-generation neural interfaces that enable bidirectional communication between peripheral nerves and assistive technologies. The project's breakthroughs are expected to advance the development of exoprostheses and exoskeletons, helping individuals regain both motor control and sensory feedback. To this end and under the IN3-Bio framework, we performed a comprehensive biocompatibility assessment of advanced materials for neural interfaces, following ISO 10993-compliant protocols.

In vitro assays using CHO cells were conducted to assess several key cytotoxic endpoints, including morphology, cell viability, cell metabolism, and genotoxicity potential, through both direct contact and extract-based exposure of the tested material (ISO 10993, ECVAM, OECD). Cytotoxicity of the next-generation implantable cuff electrode was also assessed using HaCaT cells. Alamar Blue metabolic activity measurements demonstrated that 100% cuff electrode exposure resulted in cell viability higher than 70% compared to the negative control in both cell lines, which represents the non-cytotoxicity threshold according to ISO 10993-5 guidelines. *In vitro* micronuclei testing revealed no statistically significant increase in chromosomal abnormalities, and statistical comparison with the mitomycin-C control supported the non-genotoxic profile of all tested implantable materials. Furthermore, morphological assessments confirmed no evidence of cell detachment, lysis, or structural aberrations in both cell systems for any of the materials examined in this study.

In total, our comprehensive translational strategy examined several cytotoxicity endpoints to ensure the safe, long-term integration of the tested materials into implantable neuroprosthetic systems, thereby supporting the goal of restoring mobility and sensation.

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Computational Repurposing of Approved and Investigational Drugs as Potential Inhibitors of GHRHR and Its Splice Variant SV1

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The growth hormone-releasing hormone receptor (GHRHR) is a class B1 G protein-coupled receptor (GPCR) primarily found in the pituitary gland, where it mediates the production of the growth hormone (GH). Structurally, it comprises 7 transmembrane domains (TM) connected by 3 extracellular loops (ECL) and possesses a large extracellular domain (ECD) formed by the N-terminal domain, which facilitates protein binding[1]. GHRHR has 4 alternative splicing structures, called splice variants (SV), that feature a type of naturally occurring anomaly. SV1 shares high sequence identity with the wild-type receptor; however, it lacks half of its ECD. The dysregulated activation and overexpression of proteins and their splice variants, such as GHRHR and SV1, might be implicated in uncontrolled cell proliferation, leading to oncogenesis[1,2]; both are found to be overexpressed in breast[1], ovarian[1], prostate[1,3], lung[2,4], and pancreatic[1] cancers. This process leads to cyclic adenosine monophosphate (cAMP) signalling and β-arrestin recruitment, resulting in further mitogenic activity and, consequently, tumour growth. Previous studies[2,3,4] have investigated the inhibition of GHRHR using small molecules. In the present study, we sought to repurpose clinically relevant compounds as potential GHRHR and SV1 inhibitors. Using molecular docking on GHRHR and SV1, we screened 3076 drugs provided by the DrugBank database[5], comprising 1130 approved and 1946 investigational drugs. We evaluated the compounds based on binding affinity and docking scores to identify the most promising candidates for further investigation and nanocarrier-based delivery design. Future work will include the synthesis of the nanocarrier systems for drug encapsulation and subsequent in vitro testing in cell lines that overexpress GHRHR. These findings may contribute new insights for a broader understanding of class B1 GPCRs in cancer biology and inform the development of novel strategies for tumour suppression.

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P90 - 90

A platform for natural product screening for anti-ageing bioactivities: application on plant extracts from the Greek flora

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We report here the setup of a platform for screening natural products, in order to identify anti-ageing bioactivities. Skin ageing is characterized by cell senescence, alterations in collagen and elastin networks and melanogenesis disorders, among others, and it may arise due to either intrinsic or extrinsic factors, such as ultraviolet (UV) radiation and oxidative stress. Since natural products are considered a rich source of anti-ageing molecules, our consortium selected a library containing 52 plant materials from the Greek flora and produced 104 extracts using Accelerated Solvent Extraction (ASE) and Supercritical Fluid Extraction (SFE) to be screened for a panel of biological activities related to skin ageing. In particular, the extracts were assessed for their effects on 1) human skin cell viability, 2) antioxidant activity – using both cell-free and cell-based methods – 3) photoprotective capacity, and 4) interference with collagenase, elastase, tyrosinase, as well as, with proteasomal and lysosomal activities of human skin cells. *In vivo* phenotypic screens on *Danio rerio* (zebrafish) embryos were also used for assessing melanogenesis. Many extracts with significant activities in individual assays were identified, some of them for the first time, and others in agreement with previous reports. In general, ASE extracts exhibited higher activities than SFE ones in most assays. Comparative assessment of all extracts' activities will prove valuable for the selection of specific targets for more detailed analysis. Moreover, certain extracts seem to combine multiple activities related to skin ageing. In particular, seven extracts were found to be highly effective at least in four different assays. The present study exemplifies a successful collaboration between academia and industry, supporting the potential use of these extracts against skin ageing in medicinal and cosmetic applications.

This study was co-financed by the European Regional Development Fund of the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innova-tion, under the call RESEARCH-CREATE-INNOVATE (project code: T2EDK-02583; project acronym: CosmAGE), and supported by the project "An Open-Access Research Infrastructure of Chemical Biology and Target-Based Screening Technologies for Human and Animal Health, Agriculture and the Environment (OPENSCREEN-GR)" (MIS 5002691).



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Urolithins - Metabolites with health benefits in Diabetes Mellitus

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Introduction: Urolithins are a group of metabolites from the gut and are derived from the microbial breakdown of ellagitannins, which are polyphenolic compounds commonly found in fruits and nuts. These metabolites, especially urolithin A (Uro-A) and urolithin B (Uro-B), have demonstrated important biological activities, such as antioxidant, anti-inflammatory, anti-aging and anti-cancer activities.

Aim: The main objective of this study is to investigate the role of urolithins in the development of diabetes mellitus.

Material and methods: A literature search was conducted in pubmed over the last five years.

Results: Urolithins A and B can protect against various complications of diabetes. In mice, urolithin A lowers blood sugar, improves insulin response, protects pancreatic cells and reduces oxidative stress. Urolithin B reduces fat accumulation in blood vessels, promotes cholesterol removal and protects the heart in diabetic rats. Both urolithins reduce body weight and fat mass, lower cholesterol levels and protect cells from inflammation and oxidative stress. In diabetic patients, the effect of UA on the inhibition of Akt (Protein kinase B) phosphorylation in endothelial cells may be considered as a therapeutic agent against diabetic podocytopathy by reducing ROS levels. In an E-deficient (apoE-/-) mouse model, administration of 10 mg/kg Uro B showed protective and therapeutic effects against atherosclerosis, while Uro B reduced lipid plaque deposition by inhibiting lipid uptake and cholesterol efflux in mouse and human THP-1macrophage cell lines treated with ox-LDL.

Conclusions: Urolithins are metabolites that reduce lipid uptake and promote cholesterol apoptosis. UA roles and mechanisms should be investigated in human cells, and further studies should include clinical samples.

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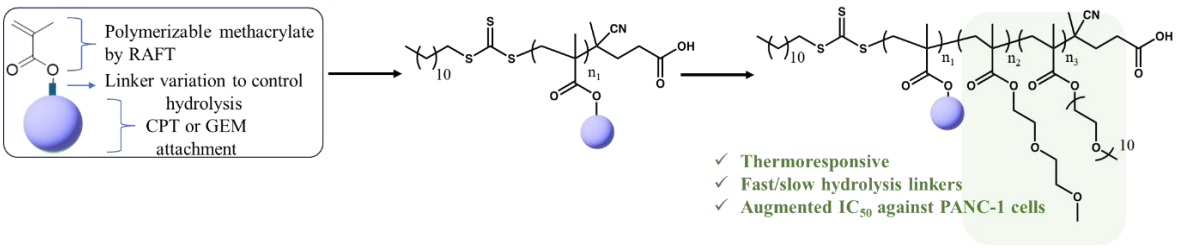
P92 - 117

Thermoresponsive block copolymer drug conjugates for targeted pancreatic cancer therapeutics

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In this work, we present a series of polymer-drug conjugates (PDCs) incorporating gemcitabine and camptothecin, linked to polymethacrylate backbones via ester and disulfide linkers. Prodrug methacrylate monomers were copolymerized with thermoresponsive oligo(ethylene glycol) methacrylate monomers to afford a series of responsive PDCs carrying multiple copies of gemcitabine and camptothecin. The PDCs could form self-assembled nanoparticles with thermoresponsive coronae. The nanoparticles showed comparable or even more potent cytotoxicity to the parent drugs against PANC-1 cancer cell line, which was further augmented above the LCST due to the hydrophobic collapse of the micelles, favouring their cellular uptake into the cytoplasm. Additional steps to enhance the cytotoxicity profile were considered by inserting sonosensitizers as reactive oxygen species generators activated by ultrasound to induce synergistic apoptotic pathways. Our proposed strategy constitutes a relatively simple nanomedicinal formulation platform that can combine sonothermal and sonochemical triggering as stimulation mechanisms to maximize therapeutic potency at the site of ultrasound treatment.

Keywords: polymer drug conjugates, thermoresponsive, nanomedicines, pancreatic cancer, targeted therapeutics



Scheme 1. General synthetic pathway towards thermoresponsive PDCs.

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P93 - 131

Physicochemical and biological properties of bio-functionalized electrospun fibrous membranes

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Electrospinning [1] is a simple, efficient, and low-cost technique for producing ultrafine polymer fibers and can be easily scaled up for industrial applications. The resulting fibrous membranes possess unique morphological and functional properties, making them highly attractive for biomedical use [2]. In this study, bio-functional polycaprolactone (PCL) fibrous membranes were prepared by encapsulation of silybin and quercetin and the physicochemical and biological properties of the composed material were investigated. These natural flavonoids are known for their strong antioxidant, anti-inflammatory, and anticancer activities [3]. The aim was to develop a fibrous membrane that combines the advantages of electrospun nanofibers with the synergistic effects of the two bioactive molecules, ensuring their stability and enabling controlled release. Membrane morphology investigation by Scanning Electron Microscopy (SEM) showed that pure PCL fibers were smooth and uniform, whereas the incorporation of the active compounds caused minor surface irregularities without significantly affecting fiber diameter. Drug encapsulation efficiency was quantified using a UV-Vis spectrophotometer, while the thermal behavior of the composite membranes was analyzed by differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA).

Cytotoxicity of the electrospun fiber mats was evaluated using the MTT assay on human embryonic kidney cells (HEK 293), and anticancer activity was assessed on human Hepatocarcinoma HepG2 cells. All fibrous membranes exhibited excellent biocompatibility, showing cell viability comparable to control samples. Moreover, PCL–Silybin and PCL–Quercetin membranes demonstrated a statistically significant reduction in cancer cell viability. In addition, the fibrous membranes showed remarkable antioxidant activity against H_2O_2 - induced oxidative stress, exhibiting increased cell survival, which can be attributed to the sustained release of the antioxidant drug. These biological properties underscore the potential of drug-loaded fibrous membranes for applications in pharmaceuticals, cosmetics, and biomedicine.

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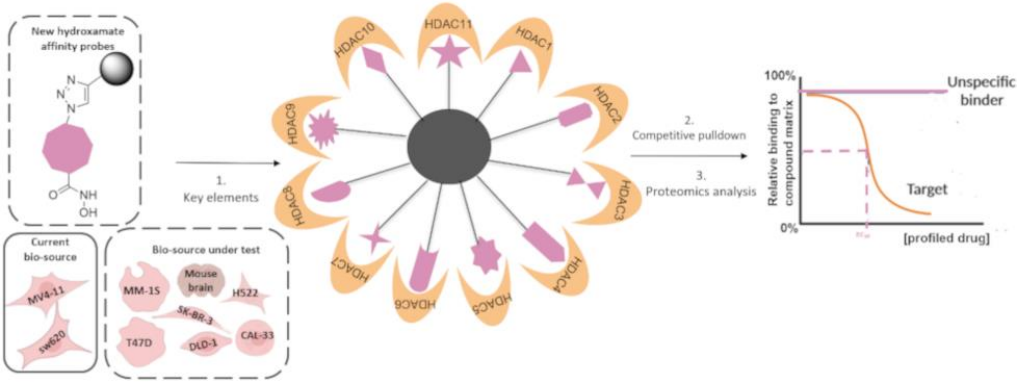
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A pan-HDAC chemoproteomics assay to profile drugs

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As some histone deacetylases (HDAC) hold key epigenetic roles, several HDAC inhibitors have already been approved for clinical use. Indeed, dysregulation of HDACs' activity leads to various pathological disorders (cancer, neurodegenerative diseases). However, the substrates of the 11 zinc-dependent HDACs are not necessarily histones, not even always proteins, and act on various post-translational acylations, sometimes with poor deacetylation activity. Understanding the biological functions and substrates of each HDAC is a major focus of ongoing research, where chemical probes discovery and chemoproteomics have a central role. Chemoproteomic approaches, like affinity-based protein profiling (AfBPP), have transformed drug target identification through the combination of small-molecule affinity probes and sensitive LC-MS/MS detection. While our team's previous efforts successfully mapped global inhibitor profiles across 9 HDACs and uncovered surprising off-targets of HDAC drugs [1,2], critical gaps remain in isoform-resolved datasets - with HDAC9 and HDAC11 remaining elusive.



Therefore, we are developing a next-generation chemoproteomic platform for pan-HDAC drug profiling. We have synthesized and tested new hydroxamate affinity probes to address the entire zinc-dependent HDAC family. Additionally, we are evaluating these molecules in different biological materials, including mouse brain and diverse human cancer cell lines, which feature various HDAC expression profiles. We particularly focus on HDAC9 and HDAC11, whose general low expression has rendered chemoproteomics analysis out of reach. With our improved assay, this research will further our understanding of the applications of HDAC inhibitors, reveal new interaction partners, and contribute to improving chemical probes and drug design. A pan-HDAC chemoproteomics assay would provide two key benefits. It would allow us to re-evaluate the actual selectivity of commonly used inhibitors. And, most importantly, it would allow us to investigate under-studied HDAC9 and HDAC11, by enhancing their enrichment and quantification in complex samples, enabling drug repurposing. Altogether, this work advances high-resolution chemoproteomic mapping of HDAC-inhibitor interactions.

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P95 - 155

Investigating a potential role for chemosensory proteins in insecticide resistance

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The use of chemical insecticides to control insect populations has led to the development of insecticide resistance. Complex insecticide formulations that include synergists, i.e. non-toxic compounds that suppress resistance, enable lower doses and longer timeframes of insecticidal compound use. Our research aims to investigate novel mechanisms that lead to resistant phenotypes, including the action of chemosensory proteins (CSPs), a unique arthropod protein family whose members have a strong affinity for aromatic compounds including most insecticide groups and are implicated in a novel resistance mechanism[1,2].

In this study, we report the functional expression of two CSPs from the fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae) and one from the mosquito *Anopheles gambiae*. Transfer vectors were constructed for baculovirus-mediated expression and recombinant bacmids were generated and transfected into *Sf9* insect cells. Using Microscale Thermophoresis (MST) binding assays with a panel of semiochemicals and insecticides from different classes with known modes of action, we detected signals indicative of ligand-binding inhibition and possible interactions of specific CSPs with compounds such as chlorfenapyr and chlorpyrifos. An AI-informed in silico screening for these interactions is also under way[3].

In parallel, using transgenic *Drosophila melanogaster* to assess resistance in vivo, we have generated transgenic lines expressing CSPs alone or in combination with cytochrome P450 enzymes involved in insecticide metabolism. This design allows us to dissect both the individual and combined contributions of candidate metabolic and sequestration mechanisms.

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P96 - 169

Elucidating the molecular mechanism underlying the antifungal activity of functionalized hyperbranched Polyethyleneimine - Copper derivatives

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The increasing prevalence of opportunistic fungal infections, driven by a growing immunocompromised population and high mortality rates, indicates the urgent need for novel antifungal agents. Existing drugs, though effective, have a limited variety of mechanisms of action, accelerating resistance development and primarily targeting actively growing cells. Our research demonstrated that nanoparticles of hyperbranched polyethyleneimine (PEI) act as antifungals against quiescent cells of the opportunistic pathogenic fungus *Aspergillus nidulans* (Gerostathis et al., 2024). To enhance the antifungal efficacy of PEI, Copper (Cu^{2+}) terminal-group functionalized derivatives were examined with varying Cu:N molar ratios, using *A. nidulans* as a model organism. Copper was selected for chelation with PEI, not only due to its intrinsic antimicrobial properties but also due to its biological relevance, as its metabolism is associated with antifungal susceptibility (Douglas & Konopka, 2019; Athanasopoulos and Sophianopoulou unpublished results).

Our results showed that PEI-Cu complexes exhibited potent fungicidal activity against fungal quiescent conidiospores and germlings, triggered reactive oxygen species (ROS) accumulation, and disrupted mitochondrial network integrity. Interestingly, these complexes have acceptable human cell cytotoxicity at concentrations and incubation times that were sufficient for the induction of their fungicidal effect (Tsiourvas et al., 2025). Furthermore, when Cu^{+2} is supplemented exogenously, it appears to modulate PEI-induced stress, partially rescuing the fungicidal and mitochondrial-fragmenting effects of PEI and possibly attenuating PEI-induced human cell cytotoxicity. Current efforts are focusing on defining an exogenous copper concentration range that preserves the antifungal potency of PEI while maintaining acceptable human cell cytotoxicity. Based on these findings, a periodical, short-term topical application on cutaneous surfaces could be suggested in future studies

The aforementioned support further investigation of PEI and PEI-Cu complexes as promising antifungals, targeting quiescent or drug-resistant fungal populations.

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P97 - 179

Papiliocin-biofunctionalized carbon nanotubes as novel antimicrobial and apoptosis-inducing nanostructures

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The emergence of antibiotic-resistant pathogens poses a major global health challenge, emphasizing the urgent need for innovative therapeutic strategies that exceed conventional antimicrobial approaches. Antimicrobial peptides (AMPs) and nanomaterials have recently gained attention as multifunctional agents exhibiting potent antibacterial and anticancer activities through targeted interactions with microbial and tumor cell membranes. In the present study, the insect-derived AMP papiliocin was cloned into pET-29c(+) vector and recombinantly expressed in *Escherichia coli* Rosetta (DE3) cells, a strain particularly suitable for the overexpression of eukaryotic proteins. Following purification via affinity chromatography, papiliocin was immobilized onto polyethylene glycol (PEG)-functionalized multiwalled carbon nanotubes (CNTs) to enhance biocompatibility, minimize proteolytic degradation and enable targeted delivery. Successful expression was verified by immunoblotting, while Fourier-transform infrared (FT-IR) spectroscopy confirmed effective peptide immobilization onto the PEG-CNTs nanocarrier. The resulting CNTs-PEG-papiliocin nanobioconjugate displayed pronounced and broad-spectrum antibacterial activity. CNTs-PEG-papiliocin achieved strong inhibition of both *Staphylococcus aureus* and *Escherichia coli* growth, with the latter showing a more pronounced susceptibility, highlighting the enhanced efficiency of the conjugate against Gram-negative bacteria. Furthermore, it significantly reduced biofilm formation by *Candida albicans*, as determined by the TCP assay. In addition, the conjugate induced apoptosis in HeLa epithelial carcinoma cells, suggesting notable anticancer potential. In conclusion, these findings elucidate the synergistic integration of AMPs with nanocarriers as a promising and versatile platform for addressing microbial resistance and advancing therapeutic nanomedicine.



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Targeting Insect Chemosensory Proteins with Small-Molecule Inhibitors to Probe Insecticide Resistance

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Chemosensory Proteins (CSPs) play diverse physiological roles, including pheromone storage and delivery, nutrient solubilization, development, and regeneration. Crucially, CSPs have recently been implicated in insecticide resistance, often showing upregulation in pest species with high resistance to compounds like pyrethroids.[1]

CSPs are compact, small, and highly dynamic proteins, and their function is based on binding and transport hydrophobic compounds. To investigate their role in insecticide resistance and identify potential competitors of insecticide binding and recognition, we performed a two-step large-scale computational screen against the CSP from the agricultural pest *Spodoptera frugiperda*. The target protein structural model was generated using AlphaFold3 (co-folded with a pheromone) and was screened against a database of three million diverse, medicinal chemistry-friendly organic compounds with specific physicochemical property ranges, using GNINA.

After clustering the top scoring compounds, we then selected a chemically diverse subset of ten thousand compounds for a second stage, using more rigorous rescoring. This involved calculations with the structure prediction program Boltz-2. Predicting each structure in the presence of each ligand, allows to calculate apparent binding affinities for each ligand. To assess the specificity of these compounds, we expanded the complex prediction process of the same library of ten thousand compounds with two homologous CSPs. The resulting Boltz-2 affinity predictions, were compared with GNINA docking scores and top compounds were subjected to visual examination, leading to the selection of a final set of compounds suitable for *in vitro* screening.

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P99 - 223

Phenoxazine derivative ST61 displays an oxidative stress-mediated cytotoxic activity against human breast cancer cells

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Aim of the current work was the biological screening of a series of compounds from the chemical library of OPENSCREEN-GR, the latter being a national research infrastructure that supports activities ranging from the collection and management of natural extracts, as well as compounds of natural and synthetic origin to the screening and evaluation of biological activity. Here, we screened for putative anticancer activities ninety-five chemically synthesized compounds, which were originally characterized as HSP90 and DKK1 inhibitors. These compounds were tested against several human cancer cell lines (epidermoid squamous carcinoma A431 cells; non-small cell lung cancer A549 cells; breast cancer MCF7 and MDA-MB-231 cells; fibrosarcoma HT1080 cells; bladder cancer T24 cells and metastatic melanoma WM266-4 cells) using the colorimetric MTT assay. Phenoxazine derivatives BS115 and ST61 were found to be the most active compounds against all cancer cell lines tested by inducing G0/G1 and G2/M cell cycle arrest, as well as apoptosis. Subsequently, the selective cytotoxic effect of ST61 towards the human hormone-dependent breast cancer cell line MCF-7 in comparison to normal human breast fibroblasts was further explored. A kinetics analysis using two multiplex immunoassays pointed HSP27 as a key molecule in the response of MCF-7 to ST61's cytotoxic effect, the phosphorylation of which was found to be regulated by p38 MAPK. ST61 resulted in the induction of much higher intracellular reactive oxygen species (ROS) levels in breast cancer cells than in normal fibroblasts. Accordingly, a higher expression of heme oxygenase 1 (HO-1) in breast cancer cells was shown compared to normal fibroblasts. Pre-incubation of the cells with the known anti-oxidant N-actetyl-cysteine led to the abrogation of ST61's cytotoxic effect, thus confirming that the main mechanism leading to ST61's anti-cancer activity is through oxidative stress; interestingly similar results were also obtained using the aggressive breast cancer cell line MDA-MB-231.

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A bacterial discovery platform uncovers small cyclic peptides that effectively disaggregate human patient-derived tau fibrils and fully rescue cognitive function in small animal models of Alzheimer's disease

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Tauopathies, including Alzheimer's disease (AD), are progressive neurodegenerative disorders characterized by pathological tau aggregation. Tau misfolds, aggregates and forms neurotoxic oligomers and fibrils impairing neuronal function. Therefore, strategies aiming at reducing the accumulation of misfolded tau aggregates are considered a promising approach for developing disease-modifying therapeutics for these conditions. In this work, we used a bacterial discovery platform to simultaneously biosynthesize a combinatorial library of cyclic oligopeptides containing millions of members and functionally screen them in an ultrahigh-throughput manner to identify the molecules that effectively rescue tau misfolding and aggregation. Through this process, we identified hundreds of hits with potential tau aggregation-inhibitory activity and then, selected the most promising cyclic peptides for chemical synthesis and subsequent evaluation. Very excitingly, we identified cyclic oligopeptides that (1) Inhibit tau aggregation and clear tau aggregates extracted from the brains of human patients with Alzheimer's disease and other tauopathies and (2) Fully reverses learning and memory deficits in fruit fly models of AD when administered both preventively and therapeutically at low doses. These results indicate that our cyclic peptides could become therapeutic candidates for AD and related tauopathies.

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EL244, a dual ATX inhibitor and PPARγ agonist, functions as an antidiabetic drug in type II prediabetic mice

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Type II diabetes melitus is a chronic metabolic condition with a prevalence of approximately 10%. Autotaxin (ATX) is a secreted lysophospholipase D that catalyzes the extracellular production of lysophosphatidic acid (LPA), a growth factor-like signaling phospholipid implicated in several diseases. In the process of drug repurposing towards the discovery of new ATX inhibitors, we found that troglitazone (TGZ), a peroxisome proliferator-activated receptor γ (PPARγ) agonist that used to serve as an antidiabetic drug, is able to inhibit ATX. Through innovative rational design and targeted synthesis new molecules were initially designed and synthesized by incorporating structural features of TGZ and known potent ATX inhibitors in one structure. Further optimization led to EL244, which was found to retain both its ATX inhibitory activity and its PPARγ agonism *in vitro*. Inhibition of ATX *in vivo* was illustrated by the drop in LPA levels upon EL244 administration. To illustrate PPARγ agonism of EL244 *in vivo* we tested its antidiabetic function, since ligands of PPARγ are known antidiabetic drugs. To this end, we induced type II prediabetes in aged male C57BL/6 mice by the administration of high-fat diet and streptozotocin, a substance that destroys insulin-producing pancreatic beta cells. Subsequent daily intraperitoneal administration of EL244 for two weeks decreased blood glucose levels and the area under curve of the glucose tolerance test (GTT), indicating that EL244 increased insulin sensitivity and reversed prediabetes. Moreover, EL244 induced the expression of PPARγ target genes in the adipose tissue, the main expressor of PPARγ. In conclusion, EL244, the first-in-class dual ATX inhibitor and PPARγ agonist, is a promising antidiabetic drug.



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Cinnamaldehyde Analogues as antibacterial agents

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The growing threat of antibiotic resistance has intensified the search for novel strategies to enhance the efficacy of existing antibacterial agents. Cinnamaldehyde, the principal bioactive component of cinnamon essential oil extracted from the stem bark of *Cinnamomum cassia*, has attracted significant attention for its broad-spectrum antibacterial activity. Structural modification of cinnamaldehyde has emerged as a promising strategy to enhance its antimicrobial efficacy, stability, and selectivity.

For the purposes of this study, different cinnamaldehyde analogues were selected, synthesized and evaluated as potential antibacterial agents. Various substitutions on the aromatic ring and alterations to the α,β-unsaturated aldehyde moiety were investigated to determine structure–activity relationships (SAR). Subsequent assessment of selected analogues against both Gram-positive and Gram-negative model microorganisms, *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa*, revealed important functionalities required for increased activities. The addition of hydroxyl and nitro functionalities at the para-position of the cinnamaldehyde aromatic ring exhibited an enhanced bioactive profile against *Escherichia coli* and *Pseudomonas aeruginosa* when compared to the parent compound, while altering the aldehyde moiety to a methyl ketone hindered *Escherichia coli* growth inhibition potencies. Interestingly, para- methoxy and bromo α,β-unsaturated methyl ketones exhibited improved *Pseudomonas aeruginosa* growth inhibition profiles. Functionalization of the cinnamaldehyde aromatic ring improved the inhibition activity of cinnamaldehyde against *Staphylococcus aureus* but the same was not observed for α,β-unsaturated methyl ketone analogues.

This study also explores the potential of selected cinnamaldehyde analogues to act synergistically with conventional antibiotics, with promising results. These findings set the basis for designing new compounds with improved activity and selectivity and highlight the potential of cinnamaldehyde derivatives as antibiotic adjuvants, offering a promising avenue for combating multidrug-resistant bacterial infections.



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Development and cytotoxic evaluation of photo-activatable hydroxamic acid analogues as potential agents for melanoma therapy

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Cancer treatment remains a major field of interest within the scientific community, since conventional therapeutic approaches often exhibit undesirable side effects and insufficient target selectivity. Photodynamic therapies exploit an external stimulus (light) along with a photo-activatable chemical compound (photosensitizer) to localize cytotoxic effects on malignant tissue, thus minimizing harm on healthy cells. Melanoma is an aggressive and frequently lethal form of cancer that continues to warrant extensive research due to the limited effectiveness of non-invasive therapeutic approaches.

Our group investigated the synthesis and cytotoxic activity of novel photo-activatable hydroxamic acid analogues on both the human melanoma cell line A375 and the human keratinocyte cell line HaCaT. Results showed that several compounds exhibited significant cytotoxic activity, indicating their potential for therapeutic applications in future cancer strategies. These findings support the development of photo-activatable hydroxamic acid analogues as promising candidates for selective melanoma therapy with reduced off-target effects.



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Comparative Analysis of Serum DPP4 Isoforms in Diabetes Mellitus and Severe Alzheimer's Disease

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Dipeptidyl peptidase 4 (DPP4) is a multifunctional protein. As a serine protease, it cleaves N-terminal dipeptides adjacent to proline or alanine. DPP4 regulates the degradation of several bioactive peptides, including the incretins glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptide (GIP), which are crucial for glucose-dependent insulin secretion and β-cell survival. In addition to GLP-1 and GIP, other DPP4 substrates -stromal cell-derived factor-1 (SDF-1), brain natriuretic peptide (BNP), eotaxin, and substance P, are increasingly recognized for their involvement in neurological and neurodegenerative disorders, including Alzheimer's disease (AD). By cleaving these peptides, DPP4 can reduce their biological activity, fully inactivate them, or significantly shorten their half-life in circulation. Inhibition of DPP4 prolongs the half-life and biological activity of these molecules. For these reasons DPP4 is an approved pharmaceutical target for Diabetes mellitus (MD) and potential pharmaceutical target for AD. This study aimed to evaluate serum DPP4 isoforms in patients with diabetes mellitus and severe AD and to compare these profiles with those of healthy individuals. Serum samples were subjected to ion-exchange chromatography (DEAE) for isoform separation. The isolated fractions were further characterized by SDS-PAGE and Western blot to assess molecular weight differences. In DM patients, DPP4 isoforms displayed both higher and lower isoelectric points compared to controls, suggesting charge alteration processes. Additionally, diabetic subject showed an increased abundance of higher molecular weight isoforms relative to health individuals. In contrast, serum from AD patients did not show major shifts in isoelectric point compared to controls. However, AD patients presented markedly altered molecular weight profiles, with most isoforms displaying higher molecular weights than those detected in healthy subjects. In conclusion, both diabetic and Alzheimer's patients exhibit distinct DPP4 isoform patterns compared with healthy individuals, in terms of isoelectric properties and molecular mass. These differences likely reflect disease associated post-translational modification or complex formation.



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Computational analysis of selected salicylidene imines for their potential as BACE-1 inhibitors

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Amyloid beta (Aβ) peptides are the primary components of amyloid plaques, one of the key hallmarks of Alzheimer's Disease (AD). Aβ peptides are formed when amyloid precursor protein (APP) is cleaved by specific enzymes called secretases. The inhibition of β-secretase, otherwise known as beta-site APP cleaving enzyme 1 (BACE-1), has been examined as a potential therapeutic approach for the treatment of AD. Schiff bases, a sub-class of imines, have been widely investigated for their potential antimicrobial, antiviral and anticancer activity. The purpose of this study is to examine a library of Schiff bases, specifically salicylidene imines, which were previously investigated for their antioxidant and cytotoxic activity, in relation to their binding affinity to BACE-1. Molecular docking was the main computational method implemented in the present study. A BACE-1 structure containing a co-crystallized ligand was obtained from Protein Data Bank (PDB) and the test ligands (salicylidene imines) were prepared with the aid of suitable computational tools. Two separate experiments were carried out in order to examine the effect of conserved water molecules (CWM) on the docking process. For that reason, several PDB structures were superimposed and water molecules discovered to interact with key binding pocket residues were kept for the subsequent experimental stages. Validation of the docking protocol was performed by re-docking of the co-crystallized ligand. The resulting binding energies and inhibition constants show that specific compounds among the library of Schiff bases examined present binding affinities which are comparable to that of the co-crystallized ligand. Most of these compounds also interact with key residues in the active site of BACE-1. Therefore, these compounds could be further investigated *in vitro*.

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P106 - 23

Impact of heat stress on the proteome of the sponge *Aplysina aerophoba*

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Sponges play a vital role in marine ecosystems, shaping habitats and supporting a diverse array of life. However, ongoing ocean warming—particularly the rising frequency and intensity of marine heatwaves—poses a substantial threat to all marine life, including the sponge *A. aerophoba*. Proteomics studies in sponges are extremely limited, especially under conditions simulating global ocean warming scenarios. We developed a novel sample preparation protocol for sponge tissues compatible with mass spectrometry analysis. The proteomic study of *A.aerophoba* yielded 1749 proteins and ortholog mapping was employed to elucidate their function. This approach revealed many proteins involved in anatomical structure development, catalytic activity, and immune response. The developed protocol was applied to investigate differential protein expression in sponges subjected to short-term thermal shock. This marks a major step toward better understanding the sponge's cellular processes and further analysis is expected to highlight key proteins impacted after rapid thermal increase. Our effort to better understand the thermal stress response and resilience mechanisms of *A.aerophoba* through proteomics may inform future efforts to predict the fate of this ecologically and pharmaceutically valuable species in a warming ocean.

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Data-driven proteomic survival modeling reveals MAPK and apoptosis-related prognostic signatures in melanoma: a TCGA-SKCM survival analysis

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Background: Therapeutic resistance and clinical heterogeneity in melanoma are strongly influenced by dysregulation of MAPK signaling and apoptotic pathways. This study aimed to identify key protein biomarkers associated with overall survival (OS) in melanoma using RPPA-based proteomic data from The Cancer Genome Atlas (TCGA-SKCM) cohort.
Methods: RPPA expression data for 282 proteins and survival records from 479 melanoma patients (TCGA-SKCM) were obtained from UCSC Xena, yielding 353 cases with complete matched datasets. Patients were stratified into high and low expression groups (median cutoff), and OS differences were assessed by Kaplan-Meier and log-rank tests ($p<0.001$). Cox proportional hazards and parametric Weibull models were applied to estimate survival functions S(t) and evaluate proportional hazards assumptions.
Results: Fifteen of 282 proteins showed a significant association with OS (log-rank $p<0.001$). The top-ranked proteins included p27 (CDKN1B), Caspase-7, 14-3-3_zeta, Axl, ERK2, Bim, 4E-BP1_pT70, CD26, c-Kit, PKC-pan, c-Met, YAP, STAT5α, fibronectin and MEK1. Survival modeling demonstrated that higher expression of p27, Caspase-7, Bim, Axl, PKC-pan, c-Met and MEK1 was associated with improved prognosis, whereas elevated levels of 14-3-3_zeta, ERK2, 4E-BP1, CD26, c-Kit, YAP and fibronectin correlated with reduced survival. Weibull models showed excellent fit (AIC range: 1.749-2.865), stable hazard ratios overtime ($SD<10^{-15}$) and conformity with proportional hazards assumptions. Predicted survival curves (up to 20,000 days) confirmed the prognostic divergence between expression groups.
Conclusion: Proteomic survival analysis identified a subset of MAPK and apoptosis-related proteins with strong prognostic significance in melanoma. Notably, high expression of p27 and Caspase-7 was associated with favorable outcomes, whereas ERK2 and 14-3-3_zeta signified increased risk. These results underscore the translational value of RPPA-based proteomics for prognostic stratification and indicate that coordinated dysregulation of MAPK and apoptotic signaling contributes to outcome variability in melanoma.

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Comprehensive NGS-Based Detection of Germline and Somatic Mutations in HRR Genes and Hematological Malignancies

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Genetic heterogeneity across cancers highlights the need for comprehensive molecular analysis to inform diagnosis, prognosis, and therapeutic decision-making. Our laboratory implements two molecular approaches: the first utilizes targeted next-generation sequencing (NGS) to characterize homologous recombination repair (HRR) genes, while the second employs third-generation sequencing for comprehensive analysis of a 137-gene panel associated with hematological malignancies. Defects in HRR pathway can lead to genomic instability and have been associated with several hereditary disorders and tumors. Tumors harboring germline or somatic variants that cause homologous recombination deficiency (HRD) are sensitive to targeted therapies, such as PARP inhibitors. This study aims to identify germline mutations conferring HRD in patients with breast, ovarian, pancreatic, and prostate cancer through high-throughput technologies. In this context, we employ NGS for a panel of 28 HRR-related genes. The detection of pathogenic variants in these genes enables appropriate genetic counseling, supports personalized clinical management, and facilitates the selection of patients eligible for PARP inhibitor therapy.

Acute Myeloid Leukemia (AML) is a hematological malignant clonal disorder characterized by proliferation of myeloid cells and inhibition of differentiation, and compared to other hematological malignancies, often presents as a clinical emergency. The diagnostic procedure for AML is therefore critical and can be performed via a number of methods, such as cytometric detection of myeloid blasts, cytogenetic analysis, and mutational profile analysis using sequencing methods to identify somatic and germline variants. A comprehensive list of known and emerging variants, which can inform patient management clinically, is detailed in the latest European Leukemia Net guidelines. We aim at developing a sequencing analysis to identify variants in hematological malignancy-related genes while improving accessibility to patients, offering reduced analysis time and cost. In this work, we describe the application of the third-generation sequencing technology, Oxford Nanopore, in the long-read sequencing of a wide panel of disease-related genes.



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Uncovering the Genetic Complexity of Rare Diseases through Whole-Exome Sequencing

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Rare diseases (RDs) are conditions with low prevalence, affecting fewer than 1 in 2000 individuals. Globally, RDs impact nearly 400 million people, including an estimated 1 million in Greece. Their extensive phenotypic and genetic heterogeneity makes accurate diagnosis particularly challenging, leaving many patients without a definitive diagnosis. Since most RDs have a genetic origin, Whole-Exome Sequencing (WES) has become a powerful tool for identifying causative variants and achieving molecular diagnoses.
In this study, Whole-Exome Sequencing was conducted on a Greek cohort of more than 400 patients with rare diseases who remained undiagnosed at the time of analysis. The cohort included both pediatric and adult individuals, representing a wide spectrum of phenotypes, including neurodevelopmental, neurological, and neuromuscular disorders, as well as growth, metabolic, cardiovascular, and skeletal abnormalities.
Different data bases and in silico tools were used for the analysis of WES data, which identified the underlying genetic cause in 34% of cases (positive result) and additionally, in 18% of cases, a Variant of Uncertain Significance (VUS) was identified (inconclusive result). Furthermore, the pipeline analysis revealed six cases where more than one genetic locus was implicated in the disease etiology. Next step is to examine the presence of these genetic loci in population data and investigate potential correlation with the severity of the phenotype. Lastly, in negative cases with family history and a strong genetic indication Whole Genome Sequencing (WGS) was performed to identify rare and common variants that could be associated with the patient's phenotype.
Identifying the genetic cause of rare genetic diseases provides scientists and clinicians with valuable insights into the underlying mechanisms and the pathology of a disease, enabling improved prediction, prevention, and treatment strategies.



P110 - 125

Deciphering the proteomic landscape of bortezomib and LB100 combination therapy in multiple myeloma

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Multiple myeloma (MM) is a malignant plasma cell disorder characterized by uncontrolled proliferation in the bone marrow and complex molecular heterogeneity. The proteasome]]inhibitor bortezomib (BTZ) is a cornerstone of MM therapy; however, resistance frequently develops, limiting its long-term efficacy. LB100, a small-molecule inhibitor of protein phosphatase 2A (PP2A), has shown promise in enhancing the efficacy of chemotherapeutic agents in several cancers by promoting cell cycle arrest and sensitizing tumor cells to stress. Yet, its molecular interplay with BTZ in MM remains poorly understood. We investigated the proteomic response of two multiple myeloma cell lines, OPM2 and XG6, treated with BTZ alone and in combination with LB100. Dose–response experiments were performed, and cells were collected across multiple concentrations of BTZ and BTZ&LB100. Sample preparation for proteomics was done using the modified SPEED protocol, as previously described[1]. Peptides were separated using the nanoElute 2 LC system during a 30-min elution gradient. Data was acquired on a timsTOF fleX mass spectrometer, using library-free Data-Independent Acquisition (DIA) Parallel Accumulation Serial Fragmentation (PASEF). Data was analysed using Fragpipe[2], using quantification with DIA-NN[3]. Differentially expressed proteins were identified using the Curve Curator[4]; this dose-resolved approach enabled the dissection of gradual molecular transitions that occur under escalating proteasome and PP2A inhibition. Pathway and functional enrichment analyses were subsequently performed to identify biological processes and signaling pathways modulated by the combined treatment. Proteomic profiling revealed distinct dose-dependent alterations in protein abundance in OPM2 cells. BTZ treatment affected various processes, while the addition of LB100 altered several of these responses. These findings indicate that combined BTZ+LB100 treatment reshapes the proteomic landscape of multiple myeloma cells in a dose-dependent manner, highlighting potential synergistic effects and providing mechanistic insight into the interaction between proteasome inhibition and PP2A modulation

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Simultaneous proteomics and metabolomics analysis in murine bone marrow cells

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Nowadays, with the growing availability of High Resolution Mass Spectrometry and computational tools for multi-omics integration, interest in combining metabolomics and proteomics is increasing. However, limited sample material often restricts such studies, especially in preclinical models. To address this issue, we designed pilot experiments to evaluate simultaneous extraction methods that obtain high-quality metabolites and proteins from a single biological sample. As samples, we used bone marrow cells as they are relevant to immunology, cardiology and cancer research. Regarding the experimental process, bone marrow cells were isolated from four C57BL/6 mice (femurs and tibias) and extracted using three different solvents (six replicates each, 1×10^6 cells): 80% methanol, 80% acetone and 100% acetone. After brief sonication and incubation at −20°C for 1h, samples were centrifuged to separate metabolite-rich supernatants and protein pellets. Metabolites were dried and stored at −80°C, while protein pellets were dissolved in pure TFA and neutralised with Tris 2M buffer solution. Proteins were then quantified by the Lowry assay, followed by reduction and alkylation with TCEP and CAA, enzymatic digestion with trypsin, and purification using stage tips before LC–MS/MS analysis. Metabolomics was performed on a Dionex UltiMate 3000 RSLC UHPLC–MaXis Impact Q-TOF MS platform, and proteomics on a nanoElute 2–timsTOF fleX MS in diaPASEF mode. Data processing was performed with TASQ and FragPipe v22.0, respectively. Technical reproducibility assessment and metabolome-proteome coverage across the different solvents showed that extraction with 80% acetone achieved the best compromise. 2832 proteins were identified, including 1121 with a coefficient of variation (CV) below 20%. 79 out of 80 targeted metabolites were detected, 41 of which with CV<30%. Advantages and limitations of each extraction method will be discussed.



P112 - 132

The Role of RNA-Binding Proteins in Regulating Axonal Growth in Peripheral Neurons

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Axon regeneration in the adult central nervous system (CNS) is severely limited, leading to permanent neuronal damage after injury. In contrast, developing CNS and mature peripheral nervous system (PNS) axons retain a strong intrinsic capacity to regenerate. While environmental factors were long considered the main cause of this discrepancy, recent studies highlight the importance of intrinsic mechanisms, such as local translation, in determining axonal survival and regrowth. Elevated translational activity observed in developing CNS and mature PNS axons has been linked to their regenerative potential, whereas the molecular constraints underlying the limited regeneration of adult CNS axons remain poorly understood. To investigate this, our team developed *ex vivo* axotomy and axoplasm enrichment assays combined with high-throughput approaches to directly assess differential axonal responses to injury. Comparative transcriptomic and proteomic analyses of mature CNS, mature PNS, and developing CNS axons revealed distinct molecular programs associated with each system's regenerative capacity and identified RNA-binding proteins (RBPs) as key regulators of the initial axonal response to injury (Triantopoulou & Vidaki, unpublished data). The proposed study aims to functionally test a subset of differentially expressed RBPs through lentiviral-mediated overexpression in PNS (dorsal root ganglion, DRG) dissociated neurons. By moving from molecular profiling to functional validation, this work seeks to identify RBPs that promote or restrict axon growth, ultimately contributing to the discovery of novel molecular targets for nervous system repair strategies.



P113 - 180

Less is more: Caloric restriction reduces anxiety-related behavior in highly anxious female mice by increasing brain energy metabolism

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Anxiety disorders are highly prevalent in modern societies, with women being twice more susceptible than men. Caloric restriction (CR), the reduction of daily food intake without malnutrition, is a beneficial dietary intervention. However, CR effects on highly anxious populations remain understudied.

Here, we subjected high anxiety-related behavior (HAB) female and male mice to a 30% 5-week CR protocol. CR elicited anxiolytic effects in female but not male HAB mice. In HAB female mice, CR also significantly altered brain energy metabolism (glycolysis, oxidative phosphorylation and fatty acid oxidation) and quality control (proteasome protein catabolism, autophagy, mitophagy) pathways in the hypothalamus, the epicentre of food intake regulation. Taken together, these data indicate that dietary interventions affect behavior in highly anxious female populations by modifying brain energy metabolism.

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P114 - 182

Proteomic analysis of the TgRANKL mouse model of osteoporosis uncovers key regulators of disease progression

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Osteoporosis, the most prevalent bone disorder, results from an imbalance between bone-forming osteoblasts and bone-resorbing osteoclasts, causing reduced bone mass, increased fragility, and a heightened risk of fractures. Receptor Activator of nuclear factor-κB Ligand (RANKL) is a key mediator in osteoclastogenesis and bone resorption, and its inhibition by the monoclonal antibody denosumab is an established therapeutic approach for osteoporosis. Our lab has previously established a transgenic mouse model that overexpresses human RANKL and develops severe osteoporosis in both sexes (TgRANKL). We had previously performed RNA-Seq in femoral bones of these mice to identify novel biomarkers.

In this study, we performed proteomic analysis in samples from femoral bone and plasma of both male and female TgRANKL and wild type (WT) mice to identify proteins which are deregulated and may serve as biomarkers in osteoporosis. In bones of TgRANKL male mice 1262 proteins were deregulated (800 upregulated, 362 downregulated), while in bones of female TgRANKL mice 1574 proteins were deregulated (946 upregulated, 628 downregulated). 716 of the differentially expressed proteins were common in both male and female mice (456 upregulated, 260 downregulated) related to translation, transport, cytoskeleton, extracellular matrix and cell migration among others. Our comparison between proteomic and RNA-Seq data demonstrated partial overlap in the upregulated genes and proteins underscoring the complementary value of integrating multi-omics approaches. Plasma proteomics revealed 31 differentially expressed proteins in males and 103 in females. 15 of them were common in both sexes and related to cancer, bone homeostasis, connective tissue neuromuscular disorders and metabolism, while 7 were common with bone proteomics results and related to extracellular matrix, bone metabolism, metabolic pathways and enzymes, iron transport and carcinogenesis. This analysis identified several molecular candidates potentially involved in the pathophysiology of osteoporosis and highlighted distinct sex-dependent mechanisms underlying the disease.

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P115 - 200

Exploring *Cistus creticus* adaptations to environmental stress via proteomic analysis

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Cistus creticus is a seasonal dimorphic Mediterranean shrub renowned for its resilience particularly in disturbed, post-wildfire environments. This species produces a resin, "ladanon", valued since antiquity for its aromatic and medicinal properties. To cope with the stressful seasonal heat and drought, its summer leaves exhibit distinct anatomical and histochemical adaptations: they become thicker and smaller, develop more trichomes to reduce water loss and accumulate higher levels of secondary metabolites compared to winter leaves.

The present study aims to compare the proteome of *C. creticus* summer leaves collected from undisturbed mountain sites with non-stressed leaves grown under optimal greenhouse conditions. Leaf samples were collected from mount Ymittos, Greece. Various protein extraction protocols were tested, with a modified trichloroacetic acid/methanol extraction coupled with a lysis buffer proving to be the most practical and effective.

Following protein reduction, alkylation, and tryptic digestion, the resulting peptides were analyzed using nanoflow high-performance liquid chromatography coupled with high-resolution mass spectrometry. Raw data obtained from Data Independent Acquisition and Parallel Accumulation Serial Fragmentation were processed using library-free DIA-NN software for protein identification and quantification. A peptide library was created using *Gossypium herbaceum*'s genome, the closest fully sequenced phylogenetic relative. Subsequent data analysis were conducted using R scripts.

Previous studies in the fire-resistant seeds of *C. creticus* highlighted some metabolic processes activated under stress. In this study we focus on leaves, where secondary metabolism is more pronounced and heat-adaptive mechanisms are more evident. Sequencing of the *C. creticus* genome will enable a deeper understanding of the molecular mechanisms underlying the distinctive adaptations of this remarkable species.



P116 - 211

Bulk Tissue Proteogenomics: Towards Construction of the First Greek National Non-Small Lung Cancer Patient Database for Precision Oncology

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Introduction: Malignancies of the lungs remain the leading cancer-related global public health burden, representing the second most prevalent type of cancer and yet accounting for the highest mortality globally. Currently, proteogenomics analysis is the mainstay tool of precision oncology and enhancing the present diagnostic and treatment approaches. This study aimed to investigate the proteogenomic profile of non-small cell lung cancer (NSCLC) tissues of a Greek patients' population to elucidate, record and analyze key molecular aspects.

Materials and Methods: Bulk tissue biopsies of patients who were subjected to bronchoscopy and diagnosed with NSCLC were prospectively collected at the Thoracic Surgery Department of "Sotiria" Chest Diseases Hospital, Athens, Greece. The study population included patients of Stages II, III and IV along with detailed records from their clinical and demographic data. Tissue samples were analyzed using in-depth liquid chromatography–tandem mass spectrometry (LC-MS/MS) based on in-house developed protocols followed by bioinformatics analysis and rigorous statistical evaluation and next generation sequencing (NGS) genomics.

Results: Bulk tumor analysis resulted in identification of an average of 932±29 proteins per sample. Significant differences were observed in expression between early and advanced stages of the disease. Functional annotation using the Gene Ontology database indicated that the majority of proteins are involved in immune and oxidative stress response regulation, metabolic adaptation, extracellular matrix organization and cell adhesion, processes closely linked to tumor invasion and metastasis.

Conclusion: This study represents the first coordinated effort to develop a proteogenomics database for Greek lung cancer patients. Herein the proteomic output of the databases is reported. The construction of a shared proteogenomics database comprises the cornerstone of precision oncology and the efforts to record molecular profiles of diverse populations need to be enhanced in order to advance the personalized treatment of NSCLC patients globally.



P117 - 235

A Hybrid Genome Sequencing and Comparative Analysis of *Listeria monocytogenes* Strains: Exploring Lineage Diversity, Virulence, and Resistance Mechanisms

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Listeria monocytogenes is a significant foodborne pathogen associated with serious outbreaks of listeriosis worldwide. In this study, three newly identified *L. monocytogenes* isolates—C5, A2D9, and A2D10—originating from dairy and clinical environments were examined through an integrated genomic approach. Using both Oxford Nanopore and Illumina sequencing platforms, complete, high-quality genome assemblies were generated and analyzed for their evolutionary and functional characteristics. Comparative genomics revealed a highly conserved core genome along with variable accessory elements that reflect ecological adaptation. Sequence typing assigned the isolates to distinct clonal complexes (ST2, ST155, and ST1), with the ST1 strain (A2D10) displaying features consistent with increased virulence. Across all genomes, numerous virulence-associated genes were identified, including the LIPI-1 pathogenicity island, while LIPI-3 was uniquely present in A2D10. Antimicrobial resistance genes such as *fosX*, *norB*, *mprF*, *lin*, and *mdrL* were conserved among strains, alongside diverse bacterial defense mechanisms, including restriction–modification and CRISPR–Cas systems. Genomic islands, prophage sequences, and other mobile elements further highlighted the genomic plasticity of these isolates. Collectively, these findings contribute to a deeper understanding of *L. monocytogenes* evolution, persistence, and virulence, supporting improved food safety monitoring and outbreak prevention strategies.



P118 - 276

Computational Analysis of Transcriptome Data and Mapping of Functional Networks in Parkinson's Disease

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Parkinson's disease (PD) is the most common neurodegenerative movement disorder. The pathophysiology is defined by a loss of dopaminergic neurons in the substantia nigra pars compacta, however recent studies suggest that the peripheral immune system may participate in PD development. Herein, we analyzed molecular insights examining RNA-seq data obtained from the peripheral blood of both Parkinson's disease patients and healthy control. Although all age and gender groups were analyzed, emphasis is given on individuals aged 50–70, the most prevalent group for Parkinson's diagnosis. The computational workflow comprises both bioinformatics analyses and machine learning processes and the yield of the pipeline includes transcripts ranked by their level of significance, which could serve as reliable genetic signatures. Classification outcomes are also examined with a focus on the significance of selected features, ultimately facilitating the development of gene networks implicated in the disease. The thorough functional analysis of the most prominent genes, regarding their biological relevance to PD, indicates that the proposed framework has strong potential for identifying blood-based biomarkers of the disease. Moreover, this approach facilitates the application of machine learning techniques to RNA-seq data from complex disorders, enabling deeper insights into critical biological processes at the molecular level.

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P119 - 280

Short-term effects of glucagon-induced catabolism on proteomic landscape of HepG2 cells.

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Hormonal regulation of metabolic pathways is well established, with insulin promoting anabolic and glucagon promoting catabolic processes.[1],[2] While the signaling mechanisms underlying glucagon-induced catabolic regulation are well characterized[2], systematic data on the corresponding protein expression profiles remain limited. A dominant concept in modern biochemistry is that metabolic pathways rely on protein interactions, which often do not form a linear string of events but instead distribute control through a network.[3] Given that, it is essential to map the protein networks in order to identify key metabolic determinants. Towards this, by applying liquid chromatography-tandem mass spectrometry (LC-MS/MS)[4] we map differences at protein expression levels upon glucagon treatment of hepatocarcinoma cell line HepG2. HepG2 cells are an established model used in pharmacological studies of hepatic metabolism.[5] We emphasize mapping proteomic landscape of pathways related to glucose homeostasis involving glycolysis, glycogen synthesis and degradation, pentose phosphate pathway, gluconeogenesis and glycophagy. Cells were analyzed six hours after treatment with glucagon in fasting and chemically induced ER stress conditions that promote glucagon's actions.[6] Our findings facilitate clustering and categorizing protein expression levels revealing significant short-term differences after hormonal induction.

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P120 - 293

In silico comparative analysis of human lymphoma transmembranome using mass spectrometry–based proteomic data at protein and peptide level

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Plasma membrane proteins play a significant role in cell functions, comprising over 30% of the cellular proteome. Moreover, these proteins can serve as accessible biomarkers and therapeutic targets, allowing tracking, progression and stage profiling in many diseases, as they reside in the cell's surface. Therefore, their research can be of great interest especially regarding different malignancies. However, several of their characteristics, such as hydrophobicity, multi-pass topology, and comparatively low abundance in relation to cytosolic proteins, lead to a systematic underrepresentation in mass spectrometry–based proteomics analyses, which inherently favor hydrophilic peptides. In addition, the limited availability of dedicated membrane protein databases further impairs comprehensive membranome profiling. Our study addresses this challenge by conducting a comparative analysis of plasma membrane proteins in three lymphoma types: **M**antle **C**ell **L**ymphoma (**MCL**), **A**naplastic **L**arge **C**ell **L**ymphoma (**ALCL**), and **H**odgkin **L**ymphoma (**HL**). Raw MS data were analyzed using MaxQuant software, and the resulting protein- and peptide-level outputs were further examined. In particular, peptide hydrophobicity was assessed through GRAVY score calculations, and the α-helices of the identified proteins were correlated with detected intensities and sequence coverage to determine whether membrane proteins face disadvantages in identification and quantitation compared with cytosolic proteins. Finally, a differential abundance analysis was performed between MCL, ALCL, and HL and their respective healthy cell counterparts (naïve mantle zone B cells, CD8+ T cells, and germinal center B cells) to investigate lymphoma-subtype-specific alterations in plasma membrane proteins levels.

Our peptide-level statistical analysis of the mass spectrometry data confirmed a significant underrepresentation of hydrophobic, membrane-derived peptides relative to the total proteome, pinpointing a key source of detection bias. Consequently, dedicated tailored enrichment and fractionation strategies are required to better investigate their potential roles in these malignancies.



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Balancing precision with plasticity: Redefining the roles of transcription factors in early cell fate specification

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Early embryogenesis requires precise regulation of cell fate specification immediately before gastrulation, yet the molecular mechanisms guiding this transition remain incompletely understood. Using *Drosophila* embryos, we uncover a previously uncharacterized regulatory mechanism that provides both stability and precision to early gene expression, highlighting how embryonic enhancers act as "mentors of the genome" to guide cell specification and differentiation. We focus on a conserved network centered on the pioneer factors (PFs) GAF and Odd-paired (Opa/ZIC3), together with Suppressor of Hairless (Su(H)/RBPJ), integrating chromatin accessibility, histone modifications, transcription factor binding, and transcriptomic datasets to dissect early enhancer function.

We identify two mechanistically distinct classes of early enhancers. The first comprises distal, Opa-dependent elements that are initially inaccessible and require Opa to overcome Su(H)-mediated repression and H3K27me3 silencing, regulating gene programs essential for post-gastrulation development. The second class consists of proximal, feedback-primed accessible enhancers that remain accessible independently of pioneer factors due to RNAPII preloading and promoter proximity. These enhancers function as autonomous regulatory hubs, maintaining accessibility and reinforcing cell identity within Polycomb-enriched chromatin. Autoregulatory PcG domains require coordinated activity of Opa, GAF, and Su(H) to drive transcription immediately prior to gastrulation.

These feedback-primed enhancers predominantly activate dorsal ectoderm genes, ensuring robust transcription under weak or heterogeneous inputs and preventing patterning errors such as ventral fate expansion or amnioserosa loss. Our results redefine pioneer factors as stabilizers of chromatin accessibility rather than initiators and reveal a dual-mode enhancer mechanism that balances transcriptional precision with developmental plasticity. This decoupling of pioneer factor binding from chromatin accessibility suggests a regulatory strategy that leverages pre-established chromatin states, inherited accessibility, or context-specific cofactors.

This mechanism represents a conserved strategy for early developmental gene regulation, offering a framework for understanding how enhancers integrate multiple regulatory inputs to stabilize cell fate decisions and guide embryonic differentiation.



P122 - 22

RNA M-Type Pseudoknot Prediction, with syntactic pattern recognition

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RNA molecules execute critical biological functions while their three-dimensional folding patterns, in secondary structures serve computational tractable approximations for functional prediction. Pseudoknots are complex motifs where nucleotides form base-pairs even beyond their respective loops, representing structurally sophisticated elements linked to catalytic activity, gene expression regulation and viral replication. Among pseudoknot classifications, M-Type structures exhibit the highest complexity, featuring four interleaved core stem-pairs, absentees from computational prediction tools and systematic research efforts.

This research gap is addressed by extending the Knotify+ platform methodology on a grammar-based prediction framework, to enable M-Type pseudoknot detection through syntactic pattern recognition enhanced by Context-Free Grammar (CFG) models. The methodology synthesizes computation linguistics with thermodynamic principles, implementing 1,296 production-rules capturing Watson-Crick and Wobble base-pairing permutations characteristics of M-Type topology. Two complementary algorithms - YAEP parsed for systematic recognition and Brute-force dynamic programming approach – generate candidate structures. Subsequently, the candidate structures are evaluated by Minimum Free Energy (MFE) and maximum base-pairing optimization criteria. The generated implementation provides bioscientists with an open-source code capable of identifying optimal configurations from RNA sequences, processing structural parameters through specialized tree-traversal algorithms. Energy calculations employ dual frameworks (Vienna RNA and pkenergy), ensuring robust thermodynamic assessment.

Testing revealed fundamental challenges in validation established databases' lack of distinct M-Type classifications, conflating pseudoknots into unified datasets. Evaluation across sequences demonstrated pattern recognition efficacy with extended sequences and vigorous recognition capabilities.

This work delivers the first dedicated computational tool for M-Type pseudoknot prediction [2], supplementing Knotify+[3], providing the research community with essential infrastructure for exploring theses elusive structural elements. The framework enables systematic investigation of occurrence in viral genomes and regulatory RNA, potentially illuminating previously uncharacterized biological functions critical for gene therapy and evolutionary studies.

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[2] Code and implementation available at: https://github.com/tigki/M_type_pseudoknots.
[3] The Knotify+ source code and implementation details are available as a public repository at: https://github.com/ntua-dslab/Knotify/releases/tag/04-Knotify+



P123 - 40

Transcriptomic analysis identifies eIF3j as a potential key regulator of Vemurafenib resistance in melanoma cells

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Melanoma represents the deadliest form of skin cancer, characterized by its high metastatic potential. Among the genetic alterations driving melanoma progression, the $BRAF^{V600E}$ mutation stands out as the most frequently observed, leading to hyperactivation of downstream signaling cascades and perturbations in translational control. While BRAF inhibitors such as Vemurafenib have revolutionized the treatment of advanced melanoma, the inevitable development of drug resistance remains a major clinical challenge. Accumulating evidence indicates that eukaryotic initiation factors (eIFs) dysregulated during skin cancer development and resistance. To follow this notion, we established Vemurafenib-resistant (VR) A375 and Sk-Mel 5 melanoma cell lines. Transcriptomic profiling through RNA-seq in the two resistant cell lines revealed dysregulation of multiple eIFs, with eIF3j levels being significantly reduced in both VR cell lines compared to the sensitive cell lines. Notably, eIF3j, a core component of the thirteen-subunit eIF3 complex, participates in multiple stages of protein synthesis, including translation initiation, termination, and ribosome recycling. Despite its importance, the contribution of eIF3j to melanoma progression and therapeutic resistance remains poorly understood. Based on these findings, we engineered melanoma cell lines with stable eIF3j overexpression to investigate its functional role. Cell proliferation was assessed using crystal violet staining assays. Translation efficiency was quantified through puromycin incorporation followed by Western blot analysis and polysome gradient fractionation. Additionally, we examined the impact of eIF3j on cellular signaling networks by Western blot detection of phosphorylated ERK1/2, AKT, and other key pathway components. Collectively, our results indicate that eIF3j modulates critical signaling networks and cap-dependent translation in vemurafenib-resistant melanoma, suggesting its potential role in therapeutic resistance mechanisms.

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Downregulation of miR-513c and let-7c as potential diagnostic markers in breast cancer

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Breast cancer is the most common malignancy and one of the leading causes of death in the female population. Early detection of the disease is of utmost importance for patient survival. This highlights the need for new molecular markers that will enhance the disease's early detection. MicroRNAs are the most widely studied representatives of sncRNAs. They act mainly as post-transcriptional gene expression regulators by binding to the mRNA-target and either suppressing its translation or causing its degradation. In the present study, total RNA was extracted from matched samples-of cancerous breast tissue and adjacent normal tissue (60 samples). Initially, the tissue samples were homogenized and the homogenates were used for total RNA extraction. Afterwards the RNA samples underwent 3'-end polyadenylation and reverse transcription using an oligo-dT adapter. Finally, the expression levels of microRNAs were quantified by an *in-house* SYBR-GREEN I real-time qPCR assay. Biostatistical analysis was performed to assess the miRNA's expression pattern and to determine its potential clinical utility in distinguishing cancer patients from healthy individuals. The results of the statistical analysis showed that both miRNAs' expression levels are downregulated in cancer tissue compared to normal adjacent tissue. Specifically, mir-513c presents a statistically significant decrease ($p<0.05$), while let-7c presents a decrease close to statistical significance ($p=0,127$). Through the analysis of sensitivity-specificity curves, the miRNAs showed a statistically significant and similar AUC index value (mir-513c=0,658, let-7c=0,650), indicating their diagnostic value. In conclusion, our findings identifyυ let-7c and mir-513c as potential diagnostic markers for breast cancer. Mir-513c expression levels in breast tissue biopsies may serve as a novel biomarker a novel biomarker or distinguishing malignant from benign lesions of the mammary gland.

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Exploring the role of DEAH-box helicase 35 (DHX35) in RNA metabolism and cancer-associated translational control

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RNA helicases are essential regulators of RNA metabolism, controlling diverse processes such as splicing, translation, ribosome biogenesis and RNA decay. The current study focuses on the evolutionarily conserved DEAH-box helicase DHX35 which shows strong differential expression across different types of tumors; with chronic myelogenous leukemia and highly metastatic lung adenocarcinoma exhibiting the highest expression levels. However, its biological function remains poorly characterized. Our preliminary data demonstrate that, depending on the cellular context, DHX35 displays variable localization, either in the cytoplasm, the nucleus or both. Specifically, immunofluorescence and subcellular fractionation analyses revealed that DHX35 predominantly localizes to the cytoplasm and associates with ribonucleoprotein complexes and 40S ribosomal subunit fractions. On the other hand, knockdown of DHX35 led decreased production of the precursor 47S rRNA and impaired rRNA processing, suggesting a potential involvement in ribosome biogenesis or rRNA stability. Furthermore, DHX35 knockdown resulted in reduction of several translation initiation factors and cyclins, both at the transcript and protein level, implying a broader impact of the helicase on translation and a direct or indirect effect on cell cycle regulation. Electrophoretic mobility shift assays indicated that DHX35 binds specifically to structured RNA substrates with free 3'-trailer sequences, including tRNAs and snoRNAs. At the moment, ongoing RIP-seq (RNA Immunoprecipitation sequencing) and CLIP-seq (Cross-linking Immunoprecipitation sequencing) analyses aim to define the RNA interactome of DHX35 and identify its direct RNA targets. Altogether, our findings suggest that DHX35 functions as a context-dependent regulator of RNA metabolism, possibly coordinating rRNA processing and ribosome assembly events with translational efficiency. These results expand our understanding of DEAH-box helicase function and establish a framework for future studies of DHX35 in cancer biology.



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Functional and clinical evaluation of RNase κ alternative transcripts in bladder cancer

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Bladder cancer (BlCa) is the 2nd most prevalent malignancy of the urinary system and is classified as non-muscle-invasive (NMIBC) and muscle-invasive (MIBC). Despite recent advancements in BlCa clinical therapeutics, bladder tumors are characterized by significant cellular and molecular heterogeneity, tackling modern precision medicine and highlighting the need for novel molecular markers to support personalized patients' management and guide clinical decision making. Herein, we have investigated the functional and diagnostic/prognostic relevance of RNase κ, a protein family implicated in human carcinogenesis, focusing on its classic transcript and var. 4, 7, 9, 14, and 20.

T24 and HTB-9 BlCa cell lines were used for functional analysis. Cell viability and proliferation rate were assessed using the MTT assay, which was also employed to determine IC_{50} values for cis-platin and gemcitabine treatments. RNA was isolated using TRI reagent and RNase κ transcripts' levels (var. 4, 9, and 14) were quantified by SYBR Green-fluorescent based RT-qPCR. A cohort of 128 BlCa patients was analyzed, with relapse/progression and mortality as clinical endpoints for NMIBC and MIBC, respectively. Survival analysis was performed by Kaplan-Meier curves and Cox regression models.

RNase κ transcripts were significantly upregulated in both cell lines after cis-platin treatment (24 & 48 h) and in T24 cells following gemcitabine treatment (72 h). In BlCa patient samples, reduced RNase κ var.9 (p=0.033) or var.14 (p=0.040) expression in MIBC correlated with higher risk of progression (relapse and/or death). Similarly, decreased expression of var.14 (p=0.028) in NMIBC was linked to increased relapse and tumor invasion risk**.** Finally, Cox regression models confirmed the prognostic impact of RNase κ downregulation in MIBC (*v.9*: HR=2.755, p=0.043; *v.14*: HR=2.203, p=0.049) and NMIBC (*v.14*: HR=2.858, p=0.037).

Reduced expression of RNase κ alternative transcripts v.9 and v.14 correlates with adverse clinical outcomes in BlCa, highlighting their potential utility in patients' prognostication.



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Circular RNA expression dynamics reveal time- and dose-dependent responses to ionizing radiation in triple-negative breast cancer cells

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Triple-negative breast cancer (TNBC) is the most aggressive breast cancer subtype, associated with high recurrence rates and limited treatment options. Ionizing radiation (IR) remains a cornerstone of TNBC management, yet the molecular mechanisms underlying variable radiation responses and radioresistance remain poorly understood. Circular RNAs (circRNAs) are stable, covalently closed non-coding RNAs that have recently emerged as key post-transcriptional regulators of gene expression and therapy response. Still, their role in radiation-induced signaling remains largely unexplored. In this study, we aimed to elucidate the time- and dose-dependent dynamics of circRNA expression following IR exposure in TNBC cells. For this purpose, three well-established TNBC cell lines, BT-20, MDA-MB-231, and MDA-MB-468, were exposed to γ-irradiation at 4 Gy and 10 Gy and harvested at 2, 9, and 24 hours post-irradiation. A panel of ten functionally relevant circRNAs involved in DNA damage response, cell cycle regulation, and transcriptional control was analyzed by quantitative real-time PCR using divergent primers, while phosphorylated p53 (Ser15) levels were assessed by Western blot. *In silico* analyses were further employed to predict miRNA targets and enriched signaling pathways. Six circRNAs, namely circABCC1, circADAM9, circFBXW7, circFOXO3, circNCOR1, and circTP53, showed dose-dependent upregulation after irradiation, displaying progressive induction that peaked at 24 hours, with circTP53 expression mirroring p53 phosphorylation. In contrast, circHIPK3, circUBAP2, circCCNB1, and circATAD2 were downregulated, showing early repression with limited recovery. Distinct expression trends among the three TNBC cell lines reflected subtype-specific heterogeneity in radiation response. Functional annotation revealed enrichment of the p53, FOXO, MAPK, and ubiquitin-mediated proteolysis pathways for selected circRNAs, underscoring their involvement in DNA repair and cell cycle regulation. These findings highlight circRNAs as dynamic regulators of the DNA damage response, with their expression tightly linked to both radiation dose and exposure time. Particularly, circTP53, circFBXW7, and circATAD2 represent promising biomarkers for predicting radiosensitivity in TNBC.



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Nanopore sequencing and CRISPR/Cas9 genome editing uncover motif-dependent m6A demethylation by ALKBH5 in breast cancer cells

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Abstract: Breast cancer remains a major global health burden. Among the emerging regulatory layers underlying its progression, post-transcriptional RNA modifications such as N6-methyladenosine (m6A) have gained prominence as key modulators of gene expression. m6A is primarily deposited within the consensus 5′-DRACH-3′ (D = A/G/T, R = A/G, H = A/C/T), however, its enzymatic removal and sequence-context dependencies in cancer remain incompletely characterized. Here, we investigated the m6A demethylase ALKBH5 and its substrate specificity in breast cancer using a combined CRISPR/Cas9 and nanopore direct RNA sequencing approach. The CRISPR/Cas9 system was used for the efficient ALKBH5 knockout (KO) in MCF-7 cells, with successful editing confirmed by targeted DNA sequencing, Western blotting, and Whole-Genome sequencing to exclude off-target events. Comparative nanopore sequencing of wild-type (WT) and KO cells enabled direct mapping of m6A sites across the transcriptome. Using m6A-calling algorithms, we observed a global increase in m6A levels following ALKBH5 KO, particularly within specific 5′-DRACH-3′ motifs, among which 5′-AGACT-3′, 5′-GGACT-3′, 5′-GGACA-3′, 5′-AGACA-3′, and 5′-TGACT-3′ were most affected. Notably, elevated methylation was also detected at distinct non-DRACH motifs (5′-AAA-3′, 5′-AAG-3′, 5′-AAT-3′, 5′-GAC-3′, 5′-GAA-3′), revealing that ALKBH5 exhibits broader sequence specificity than previously recognized. To assess the functional impact of ALKBH5 loss, qPCR was performed for canonical ALKBH5 targets including *SOX2*, *NANOG*, *KLF4*, *FOXM1*, *PODXL*, and *MYC*. Expression levels of these transcripts were significantly decreased in KO cells compared to WT, consistent with ALKBH5-mediated demethylation. However, global transcript abundance showed only a weak correlation with methylation changes, suggesting that m6A exerts a context-dependent influence on gene expression. Collectively, our results establish ALKBH5 as a dual-specificity m6A demethylase acting on both 5′-DRACH-3′ and non-DRACH motifs, underscoring its critical role in shaping the breast cancer epitranscriptome. These findings expand the current understanding of m6A regulation and highlight ALKBH5 as a potential therapeutic target in breast cancer.

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Uncovering the alternatively spliced transcripts of the human gene family encoding the lysosomal associated membrane proteins (LAMPs) in multiple myeloma cell lines

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Alternative splicing is a crucial post-transcriptional mechanism that enhances gene expression complexity by generating multiple splice variants from a single gene, thereby greatly expanding the diversity and functional versatility of the proteome. Within the lysosome-associated membrane protein gene family – comprising *LAMP1*, *LAMP2*, *LAMP3*, *CD68* (*LAMP4*), and *LAMP5* – alternative splicing gives rise to isoforms with distinct structural and functional properties that influence lysosomal dynamics, membrane trafficking, and cellular homeostasis. Dysregulation of *LAMP* expression has been associated with cancer progression; however, their splicing profile in multiple myeloma (MM) remains largely unexplored. To address this gap, we investigated alternative splicing events in the *LAMP* gene family across ten established MM cell lines. Cells were cultured under standardized conditions, and total RNA was extracted and enriched for poly(A)+ RNAs. RNA integrity was verified by capillary electrophoresis prior to library preparation. 100-bp paired-end RNA sequencing was performed on the DNBSEQ-G400 platform, and high-quality reads were aligned to the GRCh38 human reference genome using HISAT2. Expression analysis revealed that *LAMP1* and *LAMP2* were consistently and highly expressed across all MM cell lines, whereas *LAMP3* and *LAMP5* exhibited minimal or undetectable expression; *CD68* displayed moderate levels. Consequently, alternative splicing analysis focused on *LAMP1* and *LAMP2* transcripts. Using our *in-house*–developed PERL algorithm called Alternative Splicing Detection Tool (ASDT), we identified both annotated and novel splice junctions, including exon-skipping events, confirmed through visualization in the Integrative Genomics Viewer (IGV). These findings uncover a diverse landscape of *LAMP1* and *LAMP2* splice variants in MM, revealing previously uncharacterized mRNAs that may influence lysosomal biology and disease-specific cellular adaptations. This study provides comprehensive insights into the alternative splicing dynamics of the *LAMP* gene family in MM, offering a basis for future functional and clinical investigations.



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Identification and Molecular Characterization of Novel Small Non-Coding RNAs in Breast Cancer

Identification and Molecular Characterization of Novel Small Non-Coding RNAs in Breast Cancer

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Breast cancer is a highly heterogeneous disease, resulting in diverse clinical outcomes among affected women. Small non-coding RNAs (ncRNAs), critical regulators of gene expression, have provided new insights into the molecular mechanisms driving breast cancer development and progression. Advances in high-throughput sequencing technologies have enabled the discovery of novel ncRNAs with potential cancer-related functions. **Methods**: In this study, we investigated small ncRNAs in breast cancer using publicly available RNA-sequencing data from the Sequence Read Archive (SRA). Data was analyzed using sRNAbench to identify novel ncRNAs. Four high-confidence candidate ncRNAs (MT981159, MT815876, MT815885, and PX238404) were selected for experimental validation based on their high expression levels, stable secondary structure predictions, and predicted functions. Quantitative real-time PCR (qPCR) was employed to assess their expression across a panel of breast cancer cell lines representing major molecular subtypes (luminal A, luminal B, HER2-enriched, and triple-negative breast cancer), alongside the non-tumorigenic MCF10A cell line. **Results:** The expression analysis confirmed the presence of all four candidate ncRNAs across all examined cell lines. MT981159 and MT815876 were significantly downregulated in breast cancer cells compared to MCF10A, with the strongest reductions observed in luminal A and luminal B subtypes, respectively. PX238404 showed variable expression, while MT815885 exhibited the most heterogeneous pattern. Gene Ontology (GO) enrichment analysis of their predicted target genes associated these ncRNAs with key cancer-related biological processes, including transcriptional regulation, cell proliferation, and cellular stress response. **Conclusions:** This study identifies novel small ncRNAs with distinct expression patterns in breast cancer, highlighting the need for their further investigation as potential candidates for clinical application.



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Regulation and clinical significance of the m6A RNA epitranscriptome in pediatric B-cell acute lymphoblastic leukemia

Regulation and clinical significance of the m6A RNA epitranscriptome in pediatric B-cell acute lymphoblastic leukemia

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Background and aims: Childhood B-cell acute lymphoblastic leukemia (chB-ALL) is the most common pediatric malignancy (~33%) and is characterized by numerous chromosomal/genetic alterations that guide clinical decisions. Nevertheless, the current clinical exploitation on chromosomal and (epi)genetic alterations cannot fully address the heterogeneity of patients' prognosis and treatment responses. N6-methyladenosine (m6A) is the most prevalent epitranscriptomic modification in eukaryotic RNAs, and a key epitranscriptomic mechanism regulating gene expression. Herein, we investigated the expression of m6A RNA methylation complex components, including METTL3 methylatransferase and RBM15B accessory subunit, as well as FTO and ALKBH5 m6A demethylases, towards the evaluation of their clinical and prognostic significance.

Methods: Bone marrow (BM) samples from 161 chB-ALL patients at diagnosis were analyzed. Patients were treated according to ALL-BFM95 and ALL-IC BFM2009 protocols. Total RNA was extracted, and gene expression was assessed by *in-house* RT-qPCR. Patients' median follow-up was 10.3 years, while patients' relapse (DFS) and mortality (OS) were used as endpoints.

Results: *METTL3* expression was significantly reduced in patients compared to healthy controls, while *FTO* downregulation was associated with M2 BM morphology and no complete response (CR) to BFM induction treatment (day 33). Furthermore, survival analysis revealed that the loss of *RBM15B* accessory subunit and *FTO* m6A demethylase is associated with significantly higher risk for short-term relapse (for FTO) and poor overall survival (both RBM15B and FTO) of the chB-ALL patients. Interestingly, patients exhibiting concurrent loss of both *RBM15B* and *FTO* demonstrated significantly worse disease-free and overall survival intervals, while multivariate Cox regression models unveiled their independent adverse treatment outcome compared to chB-ALL clinical used markers.

Conclusion: Loss of the *FTO* m6A demethylase and the *RBM15B* accessory subunit is strongly associated with poor prognosis and treatment outcome in chB-ALL, highlighting a possible role of m6A RNA methylation machinery in chB-ALL disease progression and management.



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Circulating miRNA-seq profiling and in-depth clinical evaluation in multiple myeloma: miR-29a as a potent liquid biopsy indicator of treatment response

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The management of multiple myeloma (MM) necessitates continuous, lifelong monitoring, which traditionally relies on invasive bone marrow biopsies that can significantly impact patients' quality-of-life. In this regard, circulating miRNAs (miRNAs) have gained attention as promising minimally invasive biomarkers due to their stability in body fluids, facilitating disease early detection and monitoring. Here, plasma samples were collected from 162 patients; 6 with monoclonal gammopathy of undetermined significance (MGUS), 6 with smoldering MM (sMM), and 150 with MM and circulating small RNAs were isolated and quantified using Qubit microRNA assay kit. Subsequently, small RNA libraries were constructed from 15 MM and 12 MGUS/sMM samples and miRNA-sequencing (miR-seq) was performed in DNBSEQ-G400 sequencer. FastQ files were retrieved and bioinformatic analysis was performed. Circulating miRs were quantified through *in-house* RT-qPCR in our MM screening cohort (n=150), followed by extensive statistical and survival analyses utilizing patients' mortality and progression (death and/or relapse) as clinical endpoints. Intriguingly, miR-seq profiling identified miR-29a as the most abundant miRNA (61.4% of total counts) and revealed a statistically significant elevation in MM compared to MGUS/sMM patients. Subsequently, Kaplan-Meier curves and univariate Cox regression analysis further demonstrated that higher levels of plasma miR-29a were significantly associated with reduced overall survival (HR=4.314; p=0.001) and short-term progression (HR=2.633; p=0.001). Furthermore, multivariate Cox regression analysis further illustrated the poor overall survival (HR=4.297; p=0.001) and short-term progression (HR=2.449; p=0.001) of the MM patients with elevated miR-29a, independently of clinical established markers. Finally, multivariate models integrating circulating miR-29a along with established MM markers further strengthen their prognostic significance and ameliorate patients' stratification. Collectively, our study supports circulating miR-29a as a robust, non-invasive indicator of adverse prognostication, endorsing its potential utility for modern non-invasive precision medicine of MM patients.

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Integrative analysis of FTO m6A RNA demethylase in ovarian cancer: Functional insights and prognostic potential

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Ovarian cancer (OC) is the most lethal gynecological malignancy in developed countries, accounting for ~5% of female cancer-related mortality worldwide. In this study, we aimed to assess the role of N6-methyladenosine (m6A) and its key mediators in OC pathogenesis and prognosis. m6A is a dynamic, prevalent and reversible RNA modification that regulates gene expression by influencing multiple aspects of RNA metabolism. Within an intricate regulatory network, the m6A demethylase FTO emerges as a pivotal regulator, affecting pathways implicated in multiple diseases, including OC. For *in vitro* experiments, SK-OV-3 and OVCAR-3 cells were employed, with FTO knockdown induced by siRNA and evaluated by CCK-8 and wound healing assays. Total RNA was extracted from ovarian tumor specimens obtained from the OVCAD consortium (n=100 patients). Global m6A levels were quantified using an ELISA-based colorimetric assay, while FTO expression was assessed by RT-qPCR. TCGA-OV dataset (n=304 patients) served as validation cohort. Survival analysis was performed using disease progression and death as clinical end-points, while Bootstrap analysis was conducted for internal validation. In the screening cohorts, high m6A levels were associated with prolonged overall survival (OS) and inversely correlated with FTO mRNA levels. Importantly, FTO overexpression was linked to early disease progression and poor OS. FTO retained its prognostic significance in multivariate models, establishing FTO as an independent molecular marker of OC prognosis. Subsequent analysis of TCGA-OV dataset confirmed that FTO levels were associated with worse OS. Functionally, FTO knockdown resulted in a dose-dependent reduction in ovarian cancer cell growth and migration, with a significant decrease observed within 24h post-transfection. Conclusively, FTO has emerged as a potent regulator of cell proliferation and migration *in vitro* and serves as a significant prognostic marker in OC, highlighting its potential role in precision medicine strategies.



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Dissecting the Vault Particle: Exploring the role of Major Vault Protein and vault RNAs in melanoma progression and therapy response

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Vault particles are highly conserved and massive ribonucleoprotein complexes found exclusively in eukaryotes, composed of 78 Major Vault Protein (MVP) monomers that enclose vault RNAs (vtRNAs) and associated proteins, such as TEP1 (telomerase-associated protein 1) and PARP4 (Poly-ADP-ribose polymerase 4). Vaults are linked to various cellular processes, and their components have emerged as modulators of oncogenic signaling. Increased MVP expression has been correlated with aggressive tumor phenotypes and multidrug resistance (MDR). However, its role in melanoma onset or drug resistance remains unexplored. Here, we investigate the involvement of MVP in melanoma metastasis and resistance mechanisms. We employed melanoma cell lines representing different stages—primary (A375), potentially metastatic (WM88), and metastatic (SKMEL5)—both sensitive and resistant to the BRAF V600E inhibitor Vemurafenib. Analysis showed significant alterations in MVP and vtRNA expression in resistant cells. Stable melanoma lines overexpressing MVP were generated and showed increased drug sensitivity. Additionally, immunofluorescence and biochemical fractionation revealed shuffling of MVP between the cytoplasm and nucleus. Subsequent RT-qPCR and Western blot analysis showed that PI3K/AKT/mTOR and MAPK pathways which are critical modulators of tumor growth, survival, and proliferation, are modulated upon overexpression of MVP. Our findings suggest that MVP influences important signaling processes in melanoma and may function as a novel regulator of pathways involved in both tumor onset and acquired resistance to Vemurafenib.

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CRISPR/Cas9-mediated RPP25-like knockout affects human RNase P function and cell viability

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RPP25-Like protein (RPP25L) shares 43% sequence identity and structural conformation similarity to its orthologue RPP25, which is a key subunit of the human RNase P complex, essential for the stability of the holoenzyme and transcriptional regulation by RNA polymerases I and III. While the precise biological role of RPP25L remains unclear, its structural similarity to RPP25 suggests that it may function as an alternative protein subunit within the RNase P complex. To get additional insights on the biological significance of RPP25L we screened HAP1 cells using CRISPR/Cas9. RT-qPCR analysis revealed significant downregulation of RNase P subunits and its substrate transcripts, suggesting impaired complex activity. Functional assays, including crystal violet and resazurin assay, demonstrated that ablation of RPP25L leads to reduced proliferation and viability. Moreover, RT-qPCR and Western blot analysis revealed reduced expression of key regulators within PI3K/AKT/mTOR and MAPK pathways, which are central mediators of cell growth and survival. Finally, knockout of RPP25L leads to decreased cell motility, based on wound healing assays at different time points. These findings highlight RPP25L as a critical factor for maintaining RNase P function, cellular growth and survival which may make up for deficiencies of its RPP25 orthologue during maturation of RNA polymerases I and III transcripts.

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IN3-BIO: An Integrated Pipeline for Biocompatibility Evaluation and Repurposing of Implantable Biomaterials in Next-Generation Neural Interfaces

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Implantable biomaterials intended for use in the development of neural interfaces provoke complex biological responses. The IN3-BIO strategy was developed to ensure the safe use and biocompatibility of advanced biomaterials. Herein, we apply a comprehensive approach that combines *in silico*, *in vitro* and *in vivo* approaches to evaluate biocompatibility and minimize adverse biological responses to next-generation neural interfaces.

Data mining from scientific repositories was conducted through our *in silico* module, where natural language processing techniques were applied to extract, curate and analyse the wealth of available information. *In vitro* validation included standardized methodologies (such as the OECD 487 micronucleus assay and assays aligned with ISO 10993 guidelines) to evaluate the cytotoxic and genotoxic effects of candidate materials. The *in vivo* component of the pipeline assessed the biological response to implantation in an *Ovis aries* model through liquid chromatography–mass spectrometry (LC-MS)-based metabolomics, complemented by histological and immunohistochemical tissue analyses.

Findings highlighted notable data sparsity and scarcity in datasets related to implantable biomaterials and confirmed the biocompatible profile of the candidate materials. Metabolic alterations in key pathways were demonstrated *in vivo*, but showed no evidence of inflammation or tissue damage (also confirmed by histological and immunohistochemical analyses).

Overall, the IN3-BIO framework, integrating *in silico*, *in vitro*, and *in vivo* approaches, provides a robust and safe translation pathway for the development of new biomaterials for neural interface systems. Such a strategy enables the early-stage preclinical validation and repurposing of advanced materials for novel biomedical applications.

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Trithorax balances ISC fate decisions via Ptx1-mediated repression of EE specification

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Adult stem cells must coordinate intrinsic transcriptional programs with external signals to sustain tissue homeostasis and regenerative capacity. In the Drosophila midgut, intestinal stem cells (ISCs) produce enterocytes (ECs) or enteroendocrine (EE) cells through fate decisions governed by Notch signaling and proneural transcription factors. While the signaling framework is well characterized, the role of chromatin regulators in modulating lineage potential remains less understood. We previously showed that the Polycomb Repressive Complex 2 (PRC2) maintains the ISC pool by repressing miR-8 and preventing premature EC differentiation. Here, we show that Trithorax plays an opposing role by limiting EE specification through repression of the achaete-scute complex (AS-C) and its downstream effector Prospero. Tissue-specific RNAi and lineage tracing experiments revealed that trx-deficient stem cells proliferate normally but are biased toward EE fate. Transcriptomic and chromatin profiling of trx-depleted progenitors identified the paired-like homeobox transcription factor Ptx1 as a trx-dependent gene. Genetic experiments support a model in which Trithorax constrains EE fate commitment via Ptx1-mediated repression of the AS-C–Pros axis, acting in concert with PRC2 to ensure ISC plasticity and appropriate fate outcomes.



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Severe immunodeficiency highly affects hippocampal neurogenesis at early postnatal stages

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Immune cells interact with stem cells in various tissues across the body and this crosstalk is increasingly recognised to be important for tissue homeostasis. In the brain, the hippocampal dentate gyrus hosts a population of neural stem cells (NSCs) that remain active across development and continue to generate new neurons throughout life. Although there is evidence that immunodeficiency leads to impaired adult hippocampal neurogenesis, the underlying mechanisms remain elusive. Our study aims to unravel whether this impairment is the result of a developmental deficit or of altered micro-environmental signaling in the adult hippocampus. To this end, we conducted a comparative study at different developmental stages between immunodeficient NOD-SCID mice with defects in both innate and adaptive immunity, immunodeficient Rag1$^{-/-}$ mice with intact innate immunity, but lack of lymphocytes and control C57BL/6J mice. Our results showed that NOD-SCID mice in adulthood (P60) have significantly reduced numbers of DCX+ neuronal precursors compared to both C57BL/6J and Rag1$^{-/-}$ mice. Despite the differential cytokine expression in the serum of NOD-SCID and C57BL/6J mice, plasma replacement from adult C57BL/6J mice does not restore hippocampal neurogenesis in adult NOD-SCID mice, suggesting that there may be a developmental deficit in the formation of the neurogenic niche. Indeed, we found that NOD-SCID mice at postnatal day 30 (P30) already have impaired hippocampal neurogenesis compared to C57BL/6J and Rag1$^{-/-}$ mice. Moreover, NOD-SCID mice at P21 and P30 have reduced numbers of GFAP+/radial glia-like NSCs and SOX2+ proliferating NSCs compared to controls. Overall, these results suggest that some components of the peripheral immune system, apart from lymphocytes, are essential for the proper establishment of the hippocampal neurogenic niche. These findings expand our knowledge of the fundamental role of the immune system in the homeostasis of the nervous system and contribute to our understanding of the complex regulation of neurogenesis.



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Ectopic expression of *MCIDAS* can initiate direct reprogramming of Human Primary Astrocytes to the ependymal lineage

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Hydrocephalus is a neurological disorder characterized by enlarged ventricles filled with cerebrospinal fluid (CSF) that cannot get absorbed adequately. Surgical placement of stunts is the major approved intervention, with poor quality of life which accommodate the manifestations of the disorder but not the underlying cause.

The ventricular lining of those hydrocephalic patients has been corrupted, causing the absence of ependymal cells, which normally reside there, that under normal circumastances could propel the CSF, utilising the coordinated movement of their unique organnelles, named cilia. Previous results from our team have characterized *MCIDAS* as one of the master regulators of ependymal cell differentiation during early stages of embryogenesis. Recent published research has proved that cortical murine astrocytes can get reprogrammed to functional ependymal cells by ectopically expressing *MCIDAS* utilizing lentiviruses. In our current study, Human primary astrocytes, being a better suited model to study the kinetics of direct lineage reprogramming on, have been used. We have hypothesized that by overexpressing *MCIDAS* using Adeno associated viruses (AAVs), we could perform a more efficient and safe reprogramming of Human primary astrocytes to the ependymal lineage. Along those lines, in Hydrocephalic patients, the astrocytes residing in the ventricular sites that lack ependymal cells, seem to exhibit a reactive phenotype while forming an astrocytic scarring in those regions. In that end, we have been trying to reactivate the Human primary astrocytes, using IL-1β and TNF-α, followed by the overexpression of *MCIDAS*, to explore if we can directly reprogram those inflamed astrocytes to functional ependymal cells, which would provide physiological relevance to our study.

Treatments that target the underlying manifestation of Hydrocephalus would by far ameliorate the quality of life of those patients. Therefore, our research strongly suggests that *MCIDAS* can serve as a good candidate for new therapeutic approaches against Hydrocephalus.



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Biocompatibility of implantable plug and cuff electrodes for peripheral nervous system interface applications in patients

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Biocompatible stimulating electrodes and neural implants are in high demand to record signals and/or interconnect the brain/nervous system with external devices. In this direction, two types of electrodes were developed, cuff electrodes and plug electrodes, to allow directional interconnection of the peripheral nervous system with exoprostheses. Cuff electrodes are gold-based electrodes embedded in thermoplastic polyurethane, while plug electrodes are gold sputtered stainless steel electrodes encapsulated in a 3D printed holder. Long-term stability and biocompatibility of these electrodes is fundamental to their approval for implantation. The hemocompatibility of these electrodes was tested based on their ability to induce hemolysis or complement activation. Testing was carried out in compliance to standard protocols from the American Society for Testing and Materials (ASTM) F756-17 and F1984-99, respectively, which comply with FDA regulations. Shortly, hemolysis results in release of hemoglobin that dimerizes and exerts toxic effects, while complement activation could result in systemic inflammation. The cytocompatibility of the electrodes was tested in neuroblastoma SH-SY5Y cells differentiated with retinoic acid, a well-established model for neuronal toxicity studies, as these cells behave like "neurons". In addition, cytocompatibility was determined against human dermal fibroblasts, since implantable devices also contact the stroma. For cytocompatibility, the indirect protocol adapted from the ISO 10993 part 5 was followed, which involves the production of material extracts, according to the ISO 10993 part 12 and incubation of the cells with these extracts. Cell viability was assessed with optical microscopy and quantified with the MTT and neutral red uptake assays. Both electrodes were found biocompatible and can, thus, be used in future *in vivo* applications.

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P141 - 168

From Organoids to Barriers: Human iPSC-Derived Models of the Outer Blood–Retinal Barrier in PRPF31 Retinitis Pigmentosa

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The outer blood-retinal barrier (oBRB), formed by the retinal pigment epithelium (RPE), photoreceptor outer segments, and the underlying choroidal endothelium, is essential for maintaining retinal homeostasis and visual function. Disruption of this barrier contributes to the pathophysiology of retinal degenerative diseases, including **Retinitis Pigmentosa (RP)**. Mutations in *PRPF31*, a pre-mRNA splicing factor, are known to cause autosomal dominant RP, however, the mechanisms through which these mutations affect oBRB integrity remain poorly understood. Here, we established a **human induced pluripotent stem cell (hiPSC)-based platform** to model the oBRB in the context of *PRPF31*-associated RP. Patient-derived and isogenic control hiPSCs were differentiated into **Retinal organoids**, **RPE cells**, and **vascular endothelial cells** using optimized protocols. Each cell type was functionally and molecularly characterized to confirm identity and maturity. These components were then **assembled into a transwell co-culture system**, reconstructing the multicellular architecture of the oBRB in vitro. Ongoing work focuses on **integrating this transwell model into a microfluidic organ-on-a-chip device** to mimic physiological flow and dynamic interactions between RPE and endothelium. This approach enables quantitative analysis of barrier function, metabolite exchange, and disease-specific phenotypes in a controlled environment. Together, this platform represents a **humanized, patient-specific model** for studying oBRB dysfunction in RP, among other retinal diseases such as age-related macular degeneration (AMD) and diabetic retinopathies, and provides a foundation for future applications in disease mechanism exploration and therapeutic screening.

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Effects of *Achillea millefolium* on the physiology of red blood cells and platelets

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Yarrow (*Achillea millefolium*) is an herb used for millennia in treating haemorrhages and wounds, as according to traditional medicine it accelerates blood clotting and exhibits healing properties. The present study aims to investigate the effects of a methanolic extract from *Achillea millefolium* (MEA) on the physiology of normal red blood cells and platelets, with the objective of determining the maximum possible hemocompatible dose. 1 g *A. millefolium* was extracted in 30 ml MeOH to a final concentration of 1 mg/ml. Microvolumes were incubated along erythrocytes of healthy donors (hematocrit 2%) for 1 and 24 hours at 4°C and 37°C. Free hemoglobin levels were measured at 540 nm and the hemolysis percentage was calculated according to the formula: %hemolysis = [(Sample absorbance – Negative control absorbance) / (Positive control absorbance – Negative control absorbance) * 100]. After 24 hours of incubation, samples were microscopically examined using a thin drop smear and May-Grünwald-Giemsa staining. Osmotic fragility was tested after incubating hematocrit 45% with MEA (38,46 ng/ml) for 1 hour at 37°C, following dilution 1:100 in solutions of increasing ionic strength 0,2-0,9% and an additional 30 min incubation. Free hemoglobin was measured as described. Blood clotting time was checked on whole blood with sodium citrate anticoagulant, addition of $CaCl_2$ and extract, and incubation at 37°C with periodic inversion (30'') until a clot was visually detected.
Hemocompatibility assays showed that the MEA is hemocompatible (%hemolysis < 2%) after the first hour of incubation at concentrations below 30,3 ng/ml (4°C και 37°C), while after 24 hours at concentrations below 12,82 ng/ml (4°C και 37°C). No morphological alterations were observed on intact red blood cells after microscopic evaluation. The extract provided relative osmoprotective activity against low ionic concentrations (<0,40% NaCl). Out of the tested concentrations, 0,12 ng/ml MEA reduced blood clotting time by 29,34% (6' 43'').

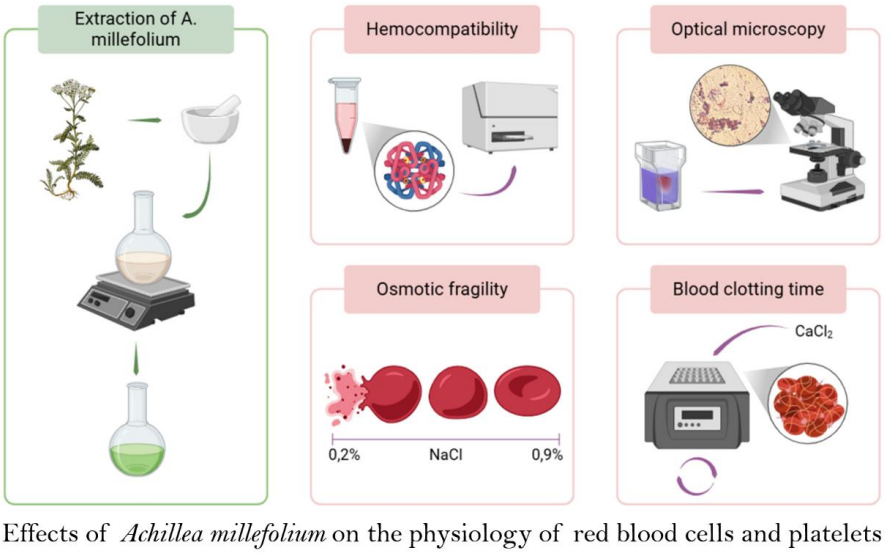


Figure 1. *Visual representation of the abstract regarding the in vitro experimental processes*

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Pharmacological inhibition of SOS1:RAS interaction promotes pancreatic β-cell regeneration

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Restoring a sufficient mass of functional β cells represents a major goal in diabetes therapy, yet achieving this through pharmacological stimulation of endogenous β-cell regeneration remains a formidable challenge. Here, we present the first pre-clinical evidence that inhibition of KRAS signaling using an orally bioavailable small molecule can directly induce β-cell regeneration and restore glucose homeostasis. KRAS, a small GTPase known primarily for its oncogenic activity, has recently been implicated in the regulation of β-cell proliferation. To dissect its functional role, we first established a mouse model expressing a constitutively active form of Kras ($Kras^{G12D}$) specifically in pancreatic endocrine cells. This genetic activation markedly impaired islet growth, leading to a significant reduction in β-cell number and islet size, confirming that hyperactive KRAS signaling negatively regulates β-cell proliferation. Conversely, pharmacological inhibition of KRAS signaling with BI-3406 - a potent, selective, and orally bioavailable SOS1:KRAS interaction inhibitor - induced robust β-cell proliferation in primary human islets, both in culture and following transplantation into immunocompromised diabetic mice. In mouse models of chemically induced diabetes, BI-3406 treatment restored β-cell mass, normalized blood glucose and insulin levels, and sustainably improved glucose tolerance. In a genetic model of type 2 diabetes ($db/db;Lepr^{db}$), BI-3406 further enhanced β-cell proliferation and maturation, resulting in persistently elevated serum insulin levels. Collectively, these findings uncover a previously unrecognized role of KRAS signaling in maintaining adult β-cell quiescence and demonstrate that targeted inhibition of this pathway can reactivate β-cell regeneration. By repurposing KRAS inhibitors already in advanced clinical development for cancer, this work opens a translationally viable avenue for regenerative diabetes therapy and establishes a new paradigm for restoring β-cell mass through targeted modulation of intracellular signaling.



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Generation of Human Pluripotent Stem Cell-Derived Populations for the Construction of a Humanized Retinal Neurovascular Unit (rNVU) Model

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The retinal neurovascular unit (rNVU), also referred to as the inner blood-retinal barrier (iBRB), is a specialized multicellular complex essential for maintaining retinal homeostasis and visual function. The iBRB is composed of retinal endothelial cells, perivascular cells, glial cells (including Müller cells and astrocytes) and neurons which interact closely to regulate vascular permeability, nutrient exchange, and neurovascular signaling. Disruption of these interactions contributes to the pathophysiology of several retinal diseases, notably diabetic retinopathy. However, current animal models fail to fully capture human-specific NVU physiology, underscoring the need for human-based systems.
In this work, we focus on the generation of the cellular populations that constitute the rNVU using human pluripotent stem cells (hPSCs). Building upon established differentiation protocols in our laboratory, we are currently producing and characterizing hPSC-derived endothelial cells and smooth muscle cells [1][2], Müller glia, obtained from our hPSC-derived retinal organoids, and astrocytes. Each population is validated through morphological, molecular, and functional assays to confirm cell identity and maturity. In parallel, we are establishing transwell-based co-culture systems to begin modeling the barrier properties of the iBRB and to evaluate cell-cell interactions under controlled conditions.
These efforts represent a crucial step toward the reconstruction of a humanized *in vitro* rNVU model. The defined hPSC-derived populations and transwell assays will together provide a robust platform for studying retinal neurovascular function, barrier integrity and disease mechanisms in a human context.

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Integrative Multi-Omics Analysis of VEGF-Driven Vasculogenesis from Human Pluripotent Stem Cells

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Regenerative Medicine (RM), leveraging stem cells immense regenerative capacity, is considered the therapy of the future. A critical hurdle is the rapid vascularization of transplanted cells, as delayed vessel formation drastically reduces cell survival. Our vascular engineering program aims to generate pre-vascularized tissue engineered constructs *in vitro* by reprogramming human fibroblasts into human induced pluripotent stem cells (hiPSCs) and subsequently differentiating them into vascular progenitor cells (VPCs) through mesodermal intermediates, leading to endothelial cells (ECs) and mural cells (MCs). Vascular Endothelial Growth Factor (VEGF-A) is crucial for endothelial cell commitment during both vasculogenesis and angiogenesis. However, the VEGF-A-induced signalling cascades that commit mesodermal VPCs during vasculogenesis are largely unknown. To address this gap, we first characterized the VEGF-responsive cell population at Day 3 (D3), termed the Mesodermal Target Population (MTP). We identified this subset by demonstrating that MTP cells expressing high VEGFR-2 levels subsequently give rise to VPCs and exhibit increased levels of the essential endothelial transcription factor ETV2. Furthermore, *in vitro* labeling of D3 mesodermal cells with a non-functional anti-VEGFR-2-Alexa488 antibody prior to VEGF-A induction showed that the resulting VPCs (CD34+ cells) harbored the internalized anti-VEGFR-2-Alexa488, confirming this subset's lineage commitment following VEGF-A stimulation.To comprehensively map the underlying molecular circuitry, we conducted single-cell RNA-sequencing (scRNA-seq) and scATAC-sequencing (Assay for Transposase-Accessible) on total D3 mesodermal population, with a secondary analysis focusing on the high VEGFR-2 expressing subset, to delineate the transcriptional and epigenomic changes driving VPC specification. Concurrently, we employed highly sensitive phosphoproteomics on the purified MTP at 5- and 15-minutes post-VEGF-A induction to pinpoint the key early phosphorylation events and activated signalling pathways. The initial datasets from these multi-omic analyses are currently undergoing processing, with subsequent steps focused on bioinformatics integration of the phosphoproteome with the scRNA-seq and scATAC-seq data to build and validate the complete regulatory networks governing human vasculogenesis. This work will close a critical gap in knowledge by defining the molecular signature of vasculogenesis, leading to improved, high-efficiency generation of vascular cells for RM applications.

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Heterogeneity of Aldh1b1^{+} ductal cells defines adult pancreatic stem cell subsets

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The existence of stem cells in the adult pancreas and their potential role in homeostatic tissue regeneration remain a matter of active debate. We have recently demonstrated that mouse ductal cells expressing the mitochondrial enzyme Aldh1b1 meet multiple criteria for classification as adult pancreatic stem cells. However, emerging evidence suggests that Aldh1b1^{+} cells are heterogeneous, as they only partially co-express other adult pancreatic stem cell markers such as Hes1 and Sox9. To investigate this heterogeneity, we performed whole-mount immunofluorescent staining of adult mouse pancreas using multiphoton microscopy, guided by duct-specific surface markers identified through meta-analysis of existing RNA-seq datasets. This revealed distinct protein expression patterns between large and small ducts, with Aldh1b1 expression restricted to the small-duct compartment. Building on these findings, we developed a multi-step FACS strategy to isolate Aldh1b1^{+} cells, sequentially enriching from total ductal cells (CD133^{+}) and then from small-duct subpopulations (Cldn8^{+}/Cldn10^{+}). We are now applying single-cell RNA-seq to this refined Aldh1b1^{+} population to identify molecular signatures that reveal its internal diversity. In parallel, we are performing spatial transcriptomics on adult pancreatic tissue sections, in order to map gene expression patterns directly within the native tissue context, preserving spatial relationships between different ductal cell types. Our goal is to distinguish functionally distinct progenitor subsets within the adult pancreatic ductal niche, ranging from multipotent to more lineage-restricted. By charting this cellular landscape, we aim to resolve the long-standing debate over the identity, potency, and regenerative role of adult pancreatic stem cells.



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Ph regulates the Indole-dependent OBP1–OBP4 heterodimer formation and dissociation in mosquito antennae

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Odorant-binding proteins (OBPs) play a crucial role in mosquito olfaction, mediating the initial step of odor recognition that guides host-seeking, foraging, mating, and oviposition behaviors [1]. OBPs undergo pH-dependent conformational changes that alter their interactions with odorants [2] and can also form heterodimers with novel binding properties [3]. *Anopheles gambiae* OBP1 and OBP4 have been found to form heterodimers, potentially contributing to the perception of indole, a human sweat attractant [4,5].
To investigate how pH modulates indole-dependent OBP1–OBP4 heterodimerization, the crystal structures of OBP4 at pH 4.6 and 8.5 were determined. Comparison with the OBP4-indole complex (3Q8I, pH 6.85) [5] revealed a flexible N-terminus and conformational changes in the α4-loop-α5 region at acidic pH. Fluorescence competition assays showed a weak indole binding to OBP4, which was further reduced at acidic pH. Differential Scanning Calorimetry (DSC) and Molecular Dynamics (MD) studies indicated that pH has a stronger effect on OBP4 stability than indole binding.
These results, combined with MD simulations of the generated OBP1-OBP4 heterodimeric models at pH 4.5, 6.5, and 8.5, suggested a possible OBP1-OBP4 heterodimer formation/disruption mechanism regulated by pH change and indole binding. The increase in pH induces the stabilization of OBP4 by enhancing its helicity, thereby enabling indole binding at neutral pH, which further stabilizes the protein and likely promotes the creation of a binding site for OBP1. The transition to the acidic environment of Odorant Receptors reduces the OBP1-OBP4 heterodimer interface stability and correlated motions, triggering its dissociation and indole release [6].
These findings indicate that local pH variations in mosquito antennae sensilla may regulate OBP interactions and odorant transport. Elucidating the molecular mechanisms governing OBP interactions not only paves the way for novel vector-control strategies but also facilitates the development of OBP-based biosensors for detecting microbial contamination indicators, like indole, in water sources.

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Structural insights into a nematode's Fatty acid & retinol carrier protein: Mi-FAR-1

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Mi-FAR-1, a retinol- and fatty acid-binding protein (FAR) in root-knot nematode *Meloidogyne incognita*, has been found to bind host-derived lipids and retinol. These interactions may influence the plant–parasite interactions and consequently affect the crop yield. However, the exact biological function of Mi-FAR-1 is not clear. In this study, we aimed to determine the structural properties of Mi-FAR-1 by experimental and computational methods.

Mi-FAR-1 protein was overexpressed in *E. coli* cells and purified by chromatography techniques. Structural determination was performed using circular dichroism (CD) spectroscopy. Three-dimensional models were generated by homology-based modeling (Swiss-Model) and artificial intelligence-based prediction (AlphaFold).

CD spectroscopy indicated that Mi-FAR-1 is in a predominantly α-helices fold with the characteristic minima at 208 and 222 nm. The protein was found to exhibit excellent thermal stability, maintaining its structure at up to 90°C with a melting temperature of 88°C, and requiring heating to 105°C for complete denaturation. Strikingly, the protein refolded upon cooling, implying partial reversible denaturation. Computational modeling supported by molecular dynamics simulations gave multiple stable 3D structures, of which one was selected as a representative model for subsequent docking studies.

Our results indicate that Mi-FAR-1 is a stable protein with a structure most likely critical to its functional role in nematode parasitism. Determining its structure and stability could facilitate identifying new means of countering *M. incognita* infection and enhancing crop protection.



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Mapping the areas of *Escherichia coli* glutaredoxin 2 used for its interaction with protein ligands

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The thioredoxin and glutaredoxin (Grx) enzyme systems of *Escherichia coli* (*E. coli*) reduce disulfides of target protein substrates in the cytosols in all living species including viruses. The Grx system is composed of EcoGrx1-4, glutathione reductase and NADPH. Although EcoGrx2 comprises up to 1 % of total soluble protein in the stationary phase of growth and has strong antioxidant properties, its function remains largely unknown. Previous work from our lab using affinity chromatography and mass spectrometry has highlighted the protein interactome of EcoGrx2. The present work applied *in silico* approaches to pinpoint the critical amino acid residues and greater surfaces (based on energy neighbor networks) for the interactions of EcoGrx2 with its protein ligands. 44 contact interfaces were identified by *in silico* docking. Residues His^{29}, Glu^{88}, Trp^{89}, Lys^{92}, Asp^{111}, Arg^{152}, Lys^{156}, Leu^{157}, Asp^{199}, Lys^{203}, Gln^{206}, and Ser^{212} were statistically favored (99 % confidence) contact residues ('contact hot spots') and coincided with 9 of the predicted 54 thermodynamic hotspots. The contact hot spots and their energy neighbors defined three major areas for interactions with protein ligands: one for covalent interactions, one for exclusively strong non-covalent interactions and one where both types of interactions occurred. All three areas were distant from the active site and occupied a rather small part of the surface area of the molecule. The largely positively charged active site area depression seemed devoid of contacts. Overall, the wider surface area of EcoGrx2 appeared not related to protein-protein interactions. It is possible that the area devoid of protein contacts may serve as a scaffold for the depression of the active site for its interaction with small negative molecules.

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Escherichia coli glutaredoxin 3: mapping of the areas of the molecule interacting with its protein ligands

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Glutaredoxin 3 (EcoGrx3) from *Escherichia coli* (*E. coli*) has been discovered in viable null mutants for the two reductors of ribonucleotide reductase Ia (RRIa), thioredoxin 1 and glutaredoxin 1. RRIa is the essential enzyme for the synthesis of deoxyribonucleotides, the precursors of DNA. EcoGrx3 may amount up to 0,5 % of total soluble protein but can barely reduce RRIa, implying that its function(s) may not be confined to the reduction of RRIa. Using affinity chromatography, we have previously identified the protein interactome of EcoGrx3. *In silico* docking of selected protein ligands with EcoGrx3, resulted in 170 interfaces for further analyses. Residues Pro^{12}, Tyr^{13}, His^{15}, Arg^{16}, Ala^{19}, Pro^{32}, Tyr^{65}, Asp^{66}, and Tyr^{69} were statistically significant (85 % confidence) contact residues ('contact hot spots'), with none of them being identical to the 59 predicted thermodynamic hotspots. If the molecule could be envisaged as a rectangle brick with the active site top right, the contact hot spots together with their energy neighbors defined a protein-protein interaction area located adjacent to the active site thiol, almost exclusively at the upper side of the brick and much less on the vertical walls of it. All types of interactions (ionic, strong non-covalent and covalent) appeared occurring in the very same area which was neutral or positively charged. The lower part of the molecule, neutral/negatively charged, may thus serve functions apparently not related to protein-protein interactions.

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Revealing Hidden Allosteric Pockets of ACE2: Structural and Functional Discovery of Potential Activators

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Introduction: Human angiotensin-converting enzyme 2 (ACE2) plays a central role in cardiovascular homeostasis by hydrolyzing the vasoconstrictor angiotensin II (Ang II: Ang 1–8) into the vasodilator angiotensin 1–7. Because of its regulatory function within the renin–angiotensin–aldosterone system, ACE2 represents an attractive therapeutic target for hypertension and related cardiovascular disorders.[1–4] Yet, very few small-molecule activators of ACE2 are known, and most display weak potency or poorly defined mechanisms[5]. This study sought to identify and characterize potential positive allosteric sites in ACE2 using structural, biochemical, and screening approaches[6].

Materials and methods: The catalytic domain of ACE2 (ACE2-CD) was expressed in *Pichia pastoris*, purified through immobilized-metal and size-exclusion chromatography following deglycosylation, and confirmed to be catalytically active. A series of phosphinic pseudopeptides, designed as transition-state analogs of Ang II, were synthesized and evaluated for binding and inhibitory properties using native substrate, a fluorogenic peptide, and thermostability assays[7].

Results: Co-crystallization of ACE2-CD with both a phosphinic pseudo-tripeptide and a phosphinic Ang II analog (p-Ang II) yielded high-resolution X-ray structures that revealed previously uncharacterized internal cavities consistent with possible allosteric binding pockets. Thermostability assays proved the rigid structure of the complexes formed between ACE2 and pseudopeptides. Complementary screening of a small FDA-approved drug library identified three compounds that enhanced ACE2 activity toward a fluorogenic pseudo-substrate[8]. Intriguingly, one of these compounds activated the enzyme under fluorogenic assay conditions but inhibited catalysis when the native Ang 1–8 substrate was used in a HPLC-based approach, suggesting a substrate-dependent mode of action or interference with the fluorescence readout.

Discussion: Together, these results demonstrate that ACE2 possesses internal structural pockets that may accommodate allosteric ligands and highlight the feasibility of identifying pharmacological activators through combined structural and functional strategies. Ongoing studies will exploit these structures for crystallization fragment-based screening and co-crystallization with activators to define the molecular basis of ACE2 activation.

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The pivotal role of Arkadia's non-RING elements in the self - and substrate - ubiquitination process

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One of the most common post-translational modifications is protein ubiquitination, which involves a highly precise three-step enzymatic cascade. Three enzymes (E1, E2 and E3) are responsible for the transfer and attachment of ubiquitin (Ub) to substrate proteins, thus determining their function and fate. E3 ubiquitin ligases are the critical components responsible for the recognition of specific substrates for ubiquitination. E3 ligase Arkadia (*RNF111*) is a positive regulator of Smad-dependent signaling via the transforming growth factor-*β* (TGF-*β*) family, that mediates the proteasome-dependent degradation of TGF-*β* suppressors i.e., inhibitory SMAD7[1], transcriptional co-repressor Ski and its close homologue SnoN (*SKIL*)[2]. It has further been reported that Arkadia effectively enhances BMP signaling via down-regulation of the BMP-specific negative regulator Smad6, thereby facilitating osteoblast differentiation[3]. However, the mechanism underlying Arkadia-mediated substrate recognition and ubiquitination remains elusive. We have previously shown that the C-terminal RING-H2 domain (residues 942–983) requires an additional N-terminal extension (non-RING elements) to activate an E2~Ub complex and promote the catalysis of Arkadia self-ubiquitination[4]. It has also been shown that these elements are essential for Arkadia's interaction with pSMAD2/3 *in vivo* [5]. Herein, we focus on the *in vitro* interaction of Arkadia with highly purified recombinant Smad6 protein. In this context, through ubiquitination assays we demonstrate that Arkadia not only interacts with but also ubiquitinates Smad6 via its C-terminal 119aa. Furthermore, using various Arkadia polypeptides we highlight the crucial role of the non-RING elements of Arkadia in Smad6 ubiquitination process. Additionally, we show that the presence of Smad6 suppresses the self-ubiquitination activity of Arkadia suggesting that self- and substrate-ubiquitination of this ligase are two competitive processes that occur within the same C-terminal region of Arkadia.

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Implications from the updated interactome of glutaredoxin 1 from *Escherichia coli*

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Living bacteria rely on redox-regulating enzymes to maintain cellular homeostasis and respond to oxidative stress. Glutaredoxin 1 (Grx1) of *Escherichia coli* is a thiol-disulfide oxidoreductase with homologues in all living species, that modulates protein function in the cytosol through reversible disulfide exchange. The protein levels of Grx1 are in strict regulation to those of ribonucleotide reductase for a balanced supply of deoxyribonucleotides used for the synthesis of DNA. This was the first discovered function for Grx1 which is also part of the antioxidant response. The current study aims at the expansion of the possible cellular roles of Grx1 by identifying its protein interactome. To this effect, wild-type Grx1, oxidized or reduced, was immobilized on affinity columns to capture interacting proteins from *E. coli* cell extracts under oxidizing and reducing conditions. Bound proteins were eluted sequentially by salt, acid and DTT and were analyzed by mass spectrometry. Strong non-covalent interactions were observed for oxidized Grx1 but not the reduced species. 16 novel ligands were discovered and added to the 15-memberd known interactome. Gene Ontology analysis of the novel ligands involved Grx1 with enzymes of central metabolism, molecular chaperones, redox-active enzymes, and ribosomal proteins. Thus, Grx1 does not only participate in classical deoxyribonucleotide synthesis and redox regulation but also in broader cellular processes such as protein folding and ribosomal assembly. In combination with our proteomic data, in silico docking suggested that reduced and oxidized Grx1 interacted with their ligands with different interfaces.

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Resurrecting Ancient Phasins: Exploring the Functional Diversity and Biotechnological Potential of Paleophasins

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Phasins are small amphiphilic proteins that act as molecular chaperones, stabilizing polyhydroxyalkanoate (PHA) granules and protecting bacterial cells from stress conditions such as heat, oxidative imbalance, osmotic and solvent stress. In this study, two ancient phasin genes ("paleo-phasins"), paleo125519 and paleo217821, - reconstructed from paleogenomic data, together with the modern phasin PhaP were heterologously expressed in *E. coli* and the respective proteins were purified. The biological activities of these proteins are being systematically investigated. Antioxidant potential is assessed using DPPH, ABTS, FRAP and DCFDA assays supported by RT-PCR analysis of antioxidant gene expression in human fibroblasts. The anti-inflammatory properties are evaluated in THP-1 monocytic cells, by quantifying pro- (IL-1β, IL-6, IL-8, TNFα) and anti-inflammatory (IL-4, IL-10) cytokine expression following LPS stimulation. Potential anticancer activity is further explored in melanoma, prostate, breast, lung and colon carcinoma preclinical models, through RT-PCR and flow cytometry to assess apoptotic markers (Caspases 3, 8, 9), early/late apoptosis, necrosis, and cell-cycle dynamics. Antioxidant evaluation of the three target phasins in a cell-free system revealed that none exhibited notable antioxidant activity, indicating a lack of significant direct antioxidant potential. All phasins demonstrated variable modulatory effects on inflammatory responses. Notably, Paleo217821 demonstrated a reduction in cell viability across three cancer cell lines, suggesting potential anticancer properties warranting further investigation. Further to this, transmission electron microscopy (TEM) was employed to characterize the induced cytotoxicity by examining ultrastructural alterations in cellular organelles of the cancer cell line in which a significant reduction in cell viability had previously been observed. Overall, this ongoing study aims to elucidate the multifunctional bioactivity of ancient phasins and to explore their biomedical and biotechnological potential.

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In Silico Structural Analysis of Ribonuclease κ Catalytic Site

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Ribonuclease κ belongs to a family of endoribonuclease proteins whose homologues are found across most metazoans. The human counterpart, referred as RNASEK, is expressed in all examined tissues and developmental stages. Initially identified as an endoribonuclease, RNASEK is associated in cellular processes like viral infection, autophagosome degradation and is involved with various types of cancer. RNASEK is a transmembrane protein and has been characterized as a subunit of the V-ATPase complex. Cryo-EM derived 3D structure reveals the presence of two transmembrane regions forming a-helixes. A loop also exists in the lumen-facing side. The gene of RNASEK is subjected to alternative splicing producing alternative splicing variants whose function is not elucidated. Taken that RNASEK participates in numerous cellular mechanisms and its interesting localization in the cell, information about the catalytic site of the enzyme could prove beneficial for determining its function and the function of the alternative splicing variants. For this purpose, molecular docking was performed with RNASEK against RNA substrate using the software HEX. The docking results revealed that the glutamate residue at site 87 (Glu87) was at the closest proximity to RNA. To verify the results 3D structures of RNASEK were reconstructed in which Glu87 was replaced by alanine and the docking was repeated. The difference between the docking energy of the mutated to the natural structure was measured. The process was repeated for residues in proximity to Glu87. The results revealed that, indeed, mutation of Glu87 causes the biggest drop in docking energy indicating importance in the interaction with the RNA substrate. Along with Glu87 the residues Asp89 and Glu91 also cause biggest reduction to the docking energy compared to the other proximal residues. The results indicate that the triad Glu87-Asp89-Glu91 could participate in the catalysis mechanism of RNASEK.

*Yazan M. Abbas et al.Structure of V-ATPase from the mammalian brain.Science**367**,1240-1246(2020).DOI:10.1126/science.aaz2924*

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Use of human natural IgG monoclonal antibodies, able to penetrate breast cancer cells and their stem cell subpopulation, as intracellular carriers of paclitaxel-loaded gold nanorods

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Background/aim: We have already shown the cell penetrating ability of polyclonal human IgGs present in Intravenous Immunoglobulin therapeutic preparations; those IgGs possessed Natural Antibodies activities, such as polyreactivity, recognizing various antigens. Herein, we isolated human monoclonal IgGs (h-mIgGs), derived from Multiple Myeloma patients' sera with high mIgG concentration, and investigated their reactivity against Tumor Associated Antigens. Moreover, we studied their penetration ability in highly aggressive MDA-MB-231 breast cancer cells and their metastatic cancer stem cell (CSC) subpopulation as well as their potential role as intracellular carriers of paclitaxel (PTX)-loaded gold nanorods (GNRs). PTX-loaded GNRs provide for the opportunity for combined chemo-photothermal cancer therapy.

Materials and Methods: Six isolated h-mIgGs were tested regarding purity by SDS-PAGE, monoclonality by IsoElectric Focusing electrophoresis (IEF) and polyreactivity by ELISA against 10 antigens including HSP90, HIF-1α, VEGF, G-actin, DNA, and TNP-BSA. The cell–penetrating ability of h-mIgGs, alone or conjugated with GNRs and GNRs-PTX, were tested by immunofluorescence (IF), and their effect on apoptosis and cell viability by FACS and MTT assay, respectively. CSC subpopulation was obtained and verified through MDA-MB-231 subculture with DMEM-F12, EGF, FGF and B-27 in non-adherent conditions and verified through IF against CD44 and CD24 stemness markers.

Results: Five h-mIgGs were cell-penetrating antibodies (CPAbs) showing cytoplasmic localization in living MDA-MB-231 cells. CPAbs penetration was more intense in CSC subpopulation. In comparison to unconjugated CPAbs, CPAbs conjugates exhibited significantly augmented intracytoplasmic IF signal. The selected CPAb35-GNRs-PTX conjugate displayed a significant decrease on MDA-MB-231 cell viability when compared to GNRs-PTX conjugates and PTX alone.

Conclusions: h-mIgGs isolated from Multiple Myeloma patients' sera can penetrate cancer cells and transport intracellularly the chemotherapeutic agent PTX conjugated to gold nanorods. Extended screening of such h-mIgGs as potential drug-carriers, particularly in resistant cancer populations such as CSC, might be an effective approach for their exploitation in biomedicine.



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Exploring in silico allosteric responses caused by cancer-associated amino acid changes in SR Protein Kinase 1

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SR protein kinases (SRPKs) catalyze the phosphorylation of SR/RS dipeptide repeats and are widely recognized as splice factor kinases that regulate various stages of mRNA splicing. PIM-1L, the long isoform of PIM-1 kinase, the splicing of which has been implicated in acute myeloid leukemia, contains a domain that consists largely of repeating SR/RS and SH/HS dipeptides (SR/SH-rich). In previous work [1], and in order to extend our knowledge on the specificity and cellular functions of SRPK1, an SRPK member, we used a combination of microsecond-scale molecular dynamics (MD) simulations combined with biochemical data, and found that the PIM-1L SR/SH-rich domain acts as a pseudo-docking peptide that binds to and inactivates SRPK1. Furthermore, comparative community network analysis of the MD trajectories enabled the identification of key SRPK1 residues mediating allosteric signal propagation within the kinase core [1]. Notably, the majority of these residues correspond to cancer-related mutations of the SRPK1 gene [2], thus validating our findings.

In the present study, we present results from an in silico quantitative energetic analysis of allosteric signaling modulation caused by the previously identified cancer-associated mutations, based on the structure-based statistical mechanical model of allostery (SBSMMA), as implemented in the AlloSigMA 2 server [3]. The MD models of SRPK1, in its apo-form and bound to the PIM-1L peptide. from our earlier work, were used for this purpose. SBSMMA assumes that allosteric signaling in proteins originates from perturbations of their equilibrium state that result in modifications of their free-energy landscape. The AlloSigMA 2 server allows exploring the nature of causality and quantification of allosteric responses in terms of per residue free energy changes upon perturbations such as ligand binding, mutations, or their combinations.

Together, this study and our previous work advance our understanding of SRPK1 regulation and its potential implications in cancer.

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Exploration of new chemical scaffolds to target glycogenolysis: the case of 7-O-methylmangiferin.

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Glycogen is the primary glucose storage in cells and a rapid and accessible form of energy that can be supplied in tissues on demand. Glycogen metabolism, synthesis and glycogenolysis, are under tight hormonal regulation while its derangements are related to serious metabolic disorders such as Glycogen Storage Diseases (GSDs)[1,2], and Diabetes Mellitus Type 2 (DM)[3]. The activity of most enzymes of glycogen metabolism is controlled by a combination of post translational modifications and allosteric effectors. In normal physiology, plasma glucose levels are controlled by intestinal absorption during the fed state, liver glycogenolysis and gluconeogenesis. In liver, inhibition of glycogenolysis is shown to reduce glucose release. Glycogen-based metabolic reprogramming has recently received renewed attention as a targetable phenotype in the tumor microenvironment[4,5]. Therefore, inhibition of glycogenolysis can be viewed under a therapeutic perspective. In previous years many compounds have been discovered to inhibit potently, but not selectively, enzymes of liver glycogenolysis mainly glycogen phosphorylase (GP)[8]. New scaffolds for selective inhibition are needed. Recent evidence has shown that natural xanthone mangiferin has a plethora of beneficial health effects such as antioxidant, antidiabetic[6] and anticancer actions[7]. In this work, we examined the effects of a mangiferin analog, 7-O-methylmangiferin (7OMF), in glycogenolysis in hepatocarcinoma cells, HepG2. Treatment of cells for 3 h with 100 μM 7OMF caused 25% inhibition of glycogenolysis which was also reflected in 31% reduction of glucose release in HepgG2 cells. Through docking experiments using the program Autodock Vina, we performed binding predictions of 7OMF in complexes with the enzyme glycogen phosphorylase and its upstream effectors phosphorylase kinase (PhK) and cAMP-dependent protein kinase A (PKA). The predicted interactions are calculated while kinetic studies are in progress.

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P160 - 213

Characterization of multifunctional enzymes

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Multifunctional proteins, capable of performing more than one distinct biological function, represent a rapidly expanding field in proteomics and structural biology. Their study offers valuable insights into the complexity of the human proteome and its multiple layers of regulation. Human multifunctional enzymes annotated with two or more Enzyme Commission (EC) numbers differing in the first digit, thus spanning distinct enzymatic classes were identified and studied further. This subset was selected both for its biological interest—proteins performing completely different catalytic functions—and for the clarity in dataset formation. The dataset, extracted from UniProt in November 2024, consisted of 142 proteins, subdivided into groups according to their specific EC class combinations (e.g., a protein annotated with EC 1.3.2.11 and 4.2.5.2 was classified as group 1;4). Each EC group was examined for internal homogeneity in protein length, family, Pfam and Clan domains, sequence, and structure. These parameters alone revealed high heterogeneity, suggesting no universal determinant of multifunctionality. However, when incorporating the classification suggested in the paper "Exploring structure-Function relationship in moonlighting proteins" by Christine Orengo , which groups multifunctional proteins according to how their catalytic sites relate across functions, a clearer pattern emerged. The proteins were classified in the following categories:

1.Distinct sites in the same domain
2. Distinct sites in different domains
3. Same residue for different functions
4. Different residues in the same/overlapping site

Extensive literature review enabled the annotation of catalytic residues for each function. Three EC combinations showed notable homogeneity: EC 1;3 (Oxidoreductases–Hydrolases), EC 2;4 (Transferases–Lyases), and EC 4;5 (Lyases–Isomerases). In all cases, the dominant category was type 3, same residue for different functions. This convergence suggests that many multifunctional enzymes achieve versatility through catalytic-site conservation, an observation that warrants further mechanistic investigation.



P161 - 285

Biochemical characterization of an alternatively spliced variant of poly(A)-specific ribonuclease (PARN) and expression profile in mesenchymal cells

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Poly(A)-specific ribonuclease, PARN, catalyzes the shortening of poly(A) tails and mediates the maturation of a broad spectrum of non-coding RNAs, including microRNAs, piRNAs, and the telomeric RNA component. We identify a splice variant of PARN, PARN_v2, notably in primary adipose-derived mesenchymal stem cells (ASCs), as well as in primary and embryonic fibroblast, and pleural malignant mesothelioma (PMM) cell lines. Phylogenetic analyses reveal an across species conservation of the novel variant, suggesting that it may bear critical biological functions and potentially belong to a "core" subclass of PARN variants. Surprisingly, we observe that PARN_v2 is catalytically active despite inherently missing two catalytical residues due to alternative splicing. We reveal residues that shape the active site of the variant and propose a cleavage mechanism combining modelling, site-directed mutagenesis and activity assays data. We explore the elusive biological roles of PARN_v2. Preliminary data revealed a correlation with differentiation states in cells of PMM subtypes that express both wild-type PARN (PARN_v1) and PARN_v2; less differentiated yet more aggressive cells express higher levels of PARN_v2, while the variant is hardly observed in benign pleural cells. We extend our search in embryonic fibroblast cells (MRC5), as well as in primary fibroblasts of lung and breast origin and ASCs. Our results show that PARN_v2 is omnipresent albeit at lower expression levels than the prevalent PARN_v1, under a variety of conditions. Ongoing research to dominantly express PARN_v2 will reveal the impact on established PARN targets and pathways.



P162 - 301

Transient interactions between the fuzzy coat and the cross-β core of brain-derived Aβ42 filaments

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Several human disorders, including Alzheimer's disease (AD), are characterized by the aberrant formation of amyloid fibrils. In many cases, the amyloid core is flanked by disordered regions, known as fuzzy coat. The structural properties of fuzzy coats, and their interactions with their environments, however, have not been fully described to date. Here, we generate conformational ensembles of two brain-derived amyloid filaments of Aβ42, corresponding respectively to the familial and sporadic forms of AD. Our approach, called metadynamic electron microscopy metainference (MEMMI), provides a characterization of the transient interactions between the fuzzy coat and the cross-β core of the filaments. These calculations indicate that the familial AD filaments are less soluble than the sporadic AD filaments, and that the fuzzy coat contributes to solubilizing both types of filament. These results illustrate how the metainference approach can help analyze cryo-EM maps for the characterization of the properties of amyloid fibrils.

Milanesi, M. Brotzakis, Z.F. Vendruscolo, M. (2025) Transient interactions between the fuzzy coat and the cross-β core of brain-derived Aβ42 filaments. Science Advances 11 (3), eadr7008.



P163 - 56

A new tension-sensitive signaling pathway involving actin polymerization prevents chromatin bridge breakage in cytokinesis

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In the presence of chromatin bridges in cytokinesis, human cells retain actin-rich structures (actin patches) at the base of the intercellular canal to prevent chromosome breakage. Here, we show that daughter nuclei connected by chromatin bridges are under mechanical tension that requires interaction of the nuclear membrane Sun1/2-Nesprin-2 Linker of Nucleoskeleton and Cytoskeleton (LINC) complex with the actin cytoskeleton, and an intact nuclear lamina. This nuclear tension promotes accumulation of Sun1/2-Nesprin-2 proteins at the base of chromatin bridges and local enrichment of the RhoA-activator PDZ RhoGEF through PDZ-binding to cytoplasmic Nesprin-2 spectrin repeats. In turn, PDZ RhoGEF activates the small GTPase RhoA and downstream ROCK-LIMK-Cofilin and mDia1 signaling to generate actin patches and prevent chromatin bridge breakage in cytokinesis. These findings identify a novel mechanosensing mechanism by which chromatin bridges promote remodeling of the actin cytoskeleton, through tension-induced activation of LINC-PDZ RhoGEF-RhoA signaling, to generate actin patches to preserve genome integrity.

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P164 - 120

WISP1-MIF axis impairs Nrf2-mediated antioxidant defence in COPD airway epithelium

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Chronic obstructive pulmonary disease (COPD) is characterised by persistent oxidative stress and impaired antioxidant defence. The matricellular protein WNT1-inducible signalling pathway protein 1 (WISP1), a downstream Wnt-pathway effector, has been implicated in tissue remodelling and inflammation. Yet its role in redox imbalance and suppression of the Nrf2 (nuclear factor E2-related factor 2) pathway in COPD remains unclear.

Primary airway epithelial cells isolated from COPD patients were treated with recombinant WISP1 (250 ng/mL, 24 h) ± the macrophage migration inhibitory factor (MIF) inhibitor ISO-1 (10 µM). Intracellular reactive oxygen species (ROS) were quantified using the 2′,7′-dichlorodihydrofluorescein diacetate (DCFH-DA) fluorescence assay. Nrf2 nuclear localisation was assessed by immunofluorescence microscopy, and Nrf2 transcriptional activity monitored via antioxidant response element (ARE)-luciferase reporter assays and qPCR of canonical target genes (HO-1, NQO1.

WISP1 induced a marked increase in intracellular ROS and concomitant suppression of Nrf2 activity. Immunofluorescence revealed reduced Nrf2 nuclear accumulation, consistent with cytoplasmic retention or enhanced nuclear export. Pharmacological inhibition of MIF with ISO-1 reversed these effects, restoring Nrf2 nuclear localisation, transcriptional activation and expression of antioxidant response genes. Both WISP1 and MIF independently elevated ROS levels, indicating a convergent mechanism promoting oxidative stress in COPD airway epithelium.

These findings identify WISP1 as a negative regulator of Nrf2-mediated antioxidant signalling and reveal a mechanistic link between WISP1 and MIF in redox homeostasis. Targeting the WISP1–MIF axis may offer a novel therapeutic strategy to restore antioxidant defences in COPD.



P165 - 302

Dissecting Transcriptional Heterogeneity in Invasive Breast Carcinoma through Spatial Transcriptomics

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Breast cancer is a highly heterogeneous disease composed of malignant cells and diverse non-cancerous cell types, including stromal and immune cells, which influence tumor growth and patient outcomes. Spatial transcriptomics allows the study of gene expression while preserving tissue architecture. In this study, we applied spatial transcriptomics to explore the intratumoral heterogeneity of the two most common breast cancer subtypes: invasive ductal carcinoma (IDC) and invasive lobular carcinoma (ILC).To enhance cellular resolution, we integrated spatial transcriptomics data with publicly available single-cell RNA sequencing (scRNA-seq) datasets obtained from the GEO (Gene Expression Omnibus) database. This integrative approach allowed precise mapping of distinct cell populations identified by scRNA-seq back onto their spatial locations within the tumor microenvironment. Through this combined analysis, we identified distinct tumor regions characterized by specific gene expression signatures, including therapy-resistant epithelial cells, multiple subsets of cancer-associated fibroblasts, and immune cell aggregates resembling tertiary lymphoid structures. These findings underscore both the spatial complexity and functional diversity within breast tumors highlighting the crucial role of the tumor microenvironment in breast cancer progression and therapy response. The integration of single-cell transcriptomics with spatial profiling provides a comprehensive view of tumor architecture and cellular interactions, essential for understanding mechanisms of resistance and immune evasion. Overall, our study supports the growing utility of spatial transcriptomics combined with single-cell genomics in refining patient stratification and advancing personalized treatment strategies in breast cancer.



P166 - 222

Fluorescent Labeling of CSC- and Bulk-Derived Extracellular Vesicles in Liver, Breast and Pancreatic Cancer

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Introduction: Extracellular vesicles (EVs) are valuable tools in drug delivery and diagnostics, but their application faces challenges, especially in isolation methods. Under the EXPAND-EV HORIZON-MSCA-2023-SE-01 project, we aim to develop reliable, versatile, and cost-effective methodologies to obtain high-purity EVs from biological samples, enabling the identification of protein differences between cancerous and normal phenotypes. For this purpose, liver, breast and pancreatic cancer cell lines (HepG2, Huh7, MDA-MB-231, Panc, BxPC3, MIA Paca-2) and immortalized human hepatocytes (IHH), were engineered to produce fluorescent EVs. Methods: Constructs encoding the mClover fluorescent protein under the control of a PGK promoter were used to label bulk cell populations, while NANOG and ALDH1A1 promoters were employed to specifically target cancer stem cell (CSC) populations. EVs from these engineered cell lines were isolated using tangential flow filtration and size exclusion chromatography (SEC). Transduction efficacy was evaluated through fluorescence and confocal microscopy while the fluorescent labeling of EVs though flow cytometry and fluorescence-activated cell sorting (FACS), followed by a colony formation assay to assess functional clonogenic potential. Results: Fluorescence and confocal microscopy images showed visible construct expression, indicating successful transduction, while flow cytometry analysis confirmed the secretion of fluorescent EVs by both cell populations. The colony assay revealed that the CSC population had increased clonogenic activity, thereby confirming the functional identity of the stem cells. Conclusions: The successful production of fluorescent EVs is a valuable tool for evaluating the efficiency of EV isolation methods. These engineered cell lines provide a reliable model for studying the release of CSC-specific extracellular vesicles across liver, breast, and pancreatic cancers. Moreover, this technique can be applied to various cancer cell types, enhancing our understanding of tumor biology and contributing to the development of improved diagnostic and therapeutic strategies.

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P167 - 149

Adipose tissue derived insulin-producing beta-like cells secrete condition media that ameliorate hyperglycemia-induced apoptosis in pancreatic β-TC6 cells

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The occurrence of Diabetes-Mellitus is one of the major challenges to human health, affecting 8.8% of the population. Up to 95% of diabetic patients suffer from Type2 Diabetes-Mellitus (T2DM). Patients exhibit progressive decrease in pancreatic β-cell mass, which is more associated with increased apoptotic β-cell death rather than reduced β-cell proliferation. Diabetes management research has shifted in recent years towards the preservation and regeneration of insulin-producing β-cells along with the prevention of early disease processes. Accordingly, agents that restore or preserve β-cell mass and function have been developed, thus slowing the progression of the disease. Therefore, a better understanding of the molecular mechanisms of islet β-cell degeneration is essential for the therapy of the disease.

In the present study, we investigated the role of secreted condition media (CM) from human Adipose Tissue-derived Insulin-Producing Beta-like Cells (AT-IPBC) on pancreatic β-cell survival. In pancreatic β-cells (β-TC6 cells), long-standing hyperglycaemia caused a reduction of β-cell nephrin expression; however, CM expoure restored nephrin expression and reduced islet β-cell apoptosis. According to our results, CM induces PI3K-dependent AKT phosphorylation which is accompanied by phosphorylation, hence inhibition, of pro-apoptotic BAD protein and suppression of FoxO1/3a transcription factors promoting pancreatic β-cell survival. Ser-136 phosphorylation of BAD appears to be the most predominant 14-3-3-binding site. Therefore, phosphorylation of BAD at Ser-136 favours binding of BAD to the scaffold protein 14-3-3 in the cytoplasm, preventing the interaction of BAD with Bcl-xL and Bcl-2 at mitochondrial membranes, thus inhibiting apoptosis.

Considering the lack of tumorigenicity of secreted conditioned media this approach may provide a valuable tool for cell therapy applications regarding diabetes. The obtained results will add advanced knowledge of the mode of CM-IPBC action on β-cell signalling and function and contribute to the development of factors which preferentially or specifically engage signalling pathways that enhance or preserve β-cell mass.



P168 - 266

Breaking the diffraction limit: Ultrastructure Expansion Microscopy enables super-resolution imaging of tiny vesicles

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Expansion microscopy (ExM) enables the physical enlargement of biological specimens, permitting super-resolution imaging with standard diffraction-limited microscopes such as conventional confocal systems. Based on the Ultrastructure Expansion Microscopy (U-ExM) protocol, established by Gambarotto et al.[1], we optimized the procedure to study the specialized secretory vesicles of human umbilical vein endothelial cells (HUVECs), known as Weibel–Palade bodies (WPBs). These organelles contribute to the normal functioning of the vascular system by releasing various bioactive molecules into the bloodstream. However, the precise sub-vesicular localization of newly identified bioactive proteins, stored in these organelles, remains challenging due to their small diameter (100–300 nm), which is below the axial resolution limit of conventional confocal microscopy (~500 nm)[2]. Consequently, conventional confocal microscopy cannot resolve the membrane from the vesicle lumen, necessitating the use of super-resolution systems to allocate the precise localization of proteins stored in these organelles. Yet, such platforms remain limited in availability due to their high cost and specialized infrastructure requirements. To overcome this limitation, we employed the technique of U-ExM. While adhering to the overall workflow of Gambarotto et al., we primarily modified the staining procedure. Reducing antibody consumption tenfold, compared to the original protocol (50µL vs. 500µL), and minimizing incubation time of secondary antibodies yielded enlarged WPBs with robust fluorescence that, for the first time, allow conventional confocal microscopy to clearly distinguish the membrane from the luminal content. Our optimizations result in a more efficient and cost-effective approach that preserves high quality labeling, warranting the use of this method in diverse systems.

To further validate our findings, we compared confocal images of expanded cells with super-resolution Structured Illumination Microscopy (SIM^2) of WPBs. In both approaches, the resolution was sufficient to distinguish the membrane from the lumen, demonstrating that our optimized U-ExM protocol enables confocal imaging at a resolution comparable to that of super-resolution microscopy for the structural analysis of secretory WPBs.

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ROCK-Myosin II pathway regulates chemotherapy-induced extracellular vesicle secretion in metastatic triple negative breast cancer

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Triple negative breast cancer (TNBC) is the most aggressive subtype of breast cancer and neoadjuvant chemotherapy (NACT) is the standard-of-care. Despite its successes, about 25% of patients with advanced disease will fail to respond or will eventually relapse, thus increasing the likelihood of metastasis. Our previous work revealed that primary tumours exposed to paclitaxel-based NACT communicate with non-cancerous stromal cells at pre-metastatic niches through the release of prometastatic extracellular vesicle (EVs), thus limiting the therapeutic efficacy of NACT. Yet, how paclitaxel induces the EV release from breast cancer cells remains unexplored. As paclitaxel stabilizes microtubules and cytoskeletal elements are known regulators of the exocytosis process, we asked whether cytoskeletal remodelling drives chemotherapy-induced EV release. Here, we show that paclitaxel treatment induces Myosin II activation through ROCK which results in a pronounced change in the cytoskeleton in a subpopulation of TNBC. Using publicly available datasets, we demonstrate that paclitaxel induces gene expression changes compatible with high ROCK-Myosin II activity. Importantly, we found that paclitaxel induces microtubule post-translational modifications, resulting in ROCK/Myosin II signaling pathway activation and enhanced release of cancer cell-derived EVs. Moreover, the released EVs are uptaken by endothelial cells in a ROCK-dependent manner. Taken together, our findings shed light on the molecular mechanism whereby paclitaxel-based NACT modulates EV release in TNBC, thus suggesting that ROCK inhibition may serve as a strategy to increase the efficacy of paclitaxel treatment in a subset of TNBC patients.



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Unraveling the role of ROCK/Myosin II in modulating the metastatic capacity of breast cancer cells

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Metastasis is considered the most common cause of cancer-related deaths and the mechanisms implicated in this process are still far from being understood. Recent studies in melanoma and pancreatic cancer have revealed that some cancer cells – particularly the ones found at the tumour invasive front - may adopt an amoeboid phenotype, which is characterized by a round morphology, enhanced actomyosin contractility and an increased number of membranous protrusions, called blebs. Cancer cells may acquire this amoeboid phenotype during local invasion and metastasis formation. Yet, the role of ROCK/Myosin II in breast cancer metastasis remains elusive. In this study, we show that the highly metastatic 4T1.2 cells display higher actomyosin contractility compared to 4T1 parental cells. To investigate whether metastatic breast cancer cells acquire an amoeboid phenotype during intravasation, we isolated circulating tumour cells (CTCs) from 4T1.GFP-tumour bearing mice, and successfully established 4T1 CTC lines from single-cell clones, These lines were further characterized for their phosphorylated myosin light chain 2 (pMLC2) status, cell circularity and number of blebs. The different CTC lines demonstrate heterogeneous amoeboid indexes and cell morphology compared to their parental 4T1.GFP$^+$ cell line, suggesting that metastatic cells may acquire different modes of epithelial-to-mesenchymal transition through different cell plasticity programs. We further show that the amoeboid phenotype of CTC lines is ROCK-dependent. Notably, high ROCK/Myosin II pathway activity promotes the metastatic capacity of both parental and CTC breast cancer cells, as ROCK inhibition ablated the number of metastases in an experimental mouse model of breast cancer metastasis. Together, our findings shed light on the cancer cell plasticity mechanisms required during breast cancer metastasis.



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In vitro electrophysiological study of cholinergic transmission in cellular and network neuronal activity along the hippocampal axis

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The hippocampus, a key component of the limbic system and hippocampal formation, plays a fundamental role in cognitive functions such as spatial navigation, learning, and memory. Its dorsal and ventral poles differ both morphologically and functionally along the dorsoventral axis. Sharp Wave - Ripple (SWR) complexes, a hallmark pattern of hippocampal neuronal activity, exhibit distinct oscillatory properties along this axis and are thought to contribute to processes such as memory consolidation and spatial navigation. The cholinergic system is critically involved in learning and memory. Fluctuations in endogenous acetylcholine levels are believed to modulate the alternation of brain rhythms between wakefulness and sleep, thereby regulating memory encoding and consolidation. In this study, we investigated the effects of cholinergic neurotransmission along the dorsoventral axis of the hippocampus. *In vitro* extracellular recordings of SWRs were obtained from the pyramidal layer of the CA1 region in transverse slices prepared from both dorsal and ventral hippocampus. To enhance endogenous acetylcholine levels, the reversible acetylcholinesterase inhibitor physostigmine was applied. Furthermore, selective antagonists of M1 and M4 muscarinic receptors were used to assess the specific contributions of these receptor subtypes and to explore potential regional differences along the hippocampal axis. Our findings demonstrate that both M1 and M4 receptors participate in the cholinergic regulation of SWRs, with their blockade producing distinct effects on various features of the hippocampal rhythm. Moreover, the modulatory effects of acetylcholine through M1 and M4 receptors were found to differ significantly between the dorsal and ventral hippocampus. These results suggest that acetylcholine exerts its regulatory actions via region-specific mechanisms, reflecting the functional specialization of the hippocampal axis.



P172 - 300

The combination of CYLD and DHX35 deficiencies induces a proteomic signature of apoptosis in a head and neck cancer cell line

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The tumor suppressor gene *Cyld* encodes a protein (CYLD) with deubiquitinase activity that has the potential to regulate several cellular homeostatic mechanisms including innate and adaptive immune responses. Bioinformatic analyses have identified *Cyld* as a component of potential synthetic lethality gene pairs in head and neck cancer. One of the highest scoring potential synthetic lethal partners of *Cyld* is *DHX35* which encodes an RNA helicase (DHX35) implicated in innate immune responses. We have demonstrated that concomitant inactivation of *Cyld* and downregulation of *DHX35* mRNA can compromise in an additive manner the viability of Cal33 and FaDu head and neck cancer cell lines. In the present study we have performed proteomic analysis of Cal33 cells and identified a correlation between the combination of CYLD and DHX35 deficiencies and the upregulation of proteins implicated in apoptosis. Our findings suggest that the combined deficiency of CYLD and DHX35 in head and neck cancer cells can sensitize them to apoptosis possibly through the engagement of innate immune signaling pathways. The molecular mechanism of this vulnerability is currently under further investigation.



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Optimization of experimental conditions for *in vitro* study of T-independent B-lymphocyte proliferation

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Lymphocyte proliferation constitutes one of the most critical functions that influence the adaptive immune response effectiveness and can be compromised in primary immunodeficiencies [1]. The *in vitro* assessment of lymphocyte proliferation is based on cell staining with carboxyfluorescein diacetate succinimidyl ester (CFSE), which, however, may affect cell viability [2]. Optimization of both CFSE staining and *in vitro* culture conditions is a key prerequisite for the reliable evaluation of the proliferative capacity and could be used for functional assessment of primary immunodeficiencies. In this study peripheral blood mononuclear cells (PBMCs) were isolated from healthy donors at the outpatient clinic of the Immunology and Histocompatibility Laboratory, University Hospital of Larissa. Cells were stained with CFSE and incubation time (10min and 20min) and temperature (20°C and 37°C) were tested. The PBMCs were subsequently cultured under different cell density, and the proliferative T-independent response of B lymphocytes was evaluated after 48h and 72h. Our data showed that the best staining conditions are (i) incubation time 10min and (ii) temperature 25°C. Enhanced B-cell proliferation was observed upon stimulation with anti-IgM and LPS, compared to anti-IgM alone. Seeding density of 3×10^5 cells/ml was associated with 41.83% divided B cells in the presence of anti-IgM ($p<0.05$) and 85.73% in the presence of anti-IgM plus LPS ($p<0.01$) 48 hours post stimulation, with no difference observed at 72h. In contrast, seeding density of 3×10^6 cells/ml, combined stimulation with anti-IgM and LPS induced a more intense proliferation after 72 hours (58.27%) compared to 48 hours (24.17%) ($p<0.05$). These findings indicate a significant impact of the staining and culture conditions on B-lymphocyte proliferation. Optimization of these parameters can provide a foundation for future functional studies of related immunopathological conditions.

Citations:

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P174 - 50

Integrin-Linked Kinase regulates Talin configuration and mechanical load distribution at muscle attachment sites

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Integrin-based adhesions to the extracellular matrix (ECM) are critical networks for mechanotransmission. A powerful model to investigate integrin mechanotransduction and quantify forces using FRET-based tension sensors in a living organism is the muscle attachment sites (MAS) of the *Drosophila* embryo, where muscles attach to epidermal tendon cells via an intervening ECM, resembling the molecular structure and function of myotendinous junctions in humans. Integrins physically connect muscle cells with the ECM. This connection requires direct binding of the extracellular domain of both integrin subunits to their ECM ligands, whereas their small cytoplasmic tails bind a network of proteins that form the integrin adhesome and mediate the link to the actin cytoskeleton. Talin is a master component within the integrin adhesome that links integrins directly to actin, enhances integrin affinity for ECM ligands, and orchestrates the recruitment of several other adhesome proteins. Moreover, structural alterations of talin protein in response to force are well documented, underlining its function as a main mechanotransmitter in integrin-based adhesions. We previously demonstrated that ILK is required to safeguard adequate immobilized integrins at the plasma membrane to sustain elevated myosin-mediated forces (Cell Reports, 2016). In *Ilk* mutants, the integrin immobile fraction remains low, and integrin-ECM adhesion weakens progressively, resulting in muscle detachment from tendons. However, we lack full understanding of how force transmission from actomyosin via talin to integrin–ligand bonds is affected in the absence of ILK. Our findings reveal a "mechanical load distribution" model in which ILK secures sufficient immobilized talin molecules, enabling effective distribution of contractile forces across multiple attachment points. Without ILK, fewer stable adhesion sites create mechanical overload on remaining connections, triggering premature sensor activation and eventual attachment failure. This work demonstrates how scaffolding proteins regulate mechanotransduction through geometric control of force-transmitting proteins.

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P175 - 150

Type II topoisomerases shape multi-scale 3D chromatin folding in regions of positive supercoils

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The spatial architecture of chromosomes plays a critical role in gene regulation and genome stability in eukaryotes. Type II topoisomerases (TOP2s) alleviate torsional stress generated during essential cellular processes and localize at chromatin loop anchors where, when trapped, they can promote genomic instability and tumorigenic chromosome fusions, as we have previously shown (*Gothe et al.*, *Mol Cell*, 2019). However, whether TOP2s relieve topological constraints at these sites or contribute more broadly to 3D chromosome folding remains unclear. Here, we combine 3D genomics, high-throughput imaging, and a newly developed method for genome-wide profiling of positive DNA supercoiling—GapRUN—to investigate the role of TOP2s in chromatin organization. Using Hi-C and Micro-C, we find that TOP2 depletion induces extensive chromatin reorganization, including the formation of new long-range contacts between active, positively supercoiled domains and lamina-associated domains (LADs), particularly during G1 phase. These changes are accompanied by altered chromatin-lamina interactions, transcriptional activity, and higher-order chromatin folding. At the loop level, TOP2 primarily influences transcriptionally anchored, positively supercoiled loops. GapRUN profiling reveals the accumulation of positive supercoils at transcriptionally active regions and at chromatin loop anchors, especially those mediating enhancer–promoter interactions. Moreover, positive supercoiling is enriched at LAD/non-LAD boundaries, where TOP2 activity modulates chromatin interactions, suggesting that TOP2s are integral to chromosome organization across multiple scales. Together, our study provides the first comprehensive map of positive DNA supercoiling in human cells and links TOP2-dependent supercoil regulation to the spatial architecture of the genome (*Longo et al.*, *Mol Cell*, 2024).



P176 - 151

Development of methods to study positive DNA supercoiling dynamics and regulation in human cells

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The topological properties of DNA, such as accumulation of positive or negative DNA supercoiling, are intimately connected with essential biological processes, such as gene expression, replication, recombination, and chromosome segregation. While negative supercoiling has been extensively characterized, positive supercoiling remains largely unexplored, due to the absence of molecular tools that can directly mark and visualize this topological state in eukaryotic genomes. We have recently utilised GAPR, a bacterial protein that selectively binds positively supercoiled DNA *(Guo et al., Cell, 2018)*, to develop GapRUN—a genome-wide profiling technique that uses the CUT&RUN approach to identify genome-wide sites of GAPR binding in the human genome. Using GapRUN, we have generated the first profile of positive DNA supercoiling in human cells and uncovered dynamic changes linked to TOP2 activity, highlighting a connection between DNA topology and chromatin organization (*Longo et al.*, *Mol Cell*, *2024)*. These results lay the foundation for a broader application of GAPR as an adaptable marker to interrogate DNA topology across multiple experimental contexts. We are currently using GAPRUN to identify how the positive DNA supercoiling landscape is influenced by changes in transcription, replication and/or chromosome organization and how misregulation promotes genomic instability. Moreover, we aim to expand the utility of GAPR by combining it with microscopy-based approaches and CRISPR-based genetic screens to uncover previously unrecognized factors of supercoiling regulation. By performing proximity labeling proteomics experiments we aim to identify regulators and/or markers of positive supercoiling in human cells, with the goal to provide the first endogenous factor capable of recognizing this feature in its native context. Together, this work establishes GAPR as a versatile tool to chart, visualize, and dissect positive DNA supercoiling, opening new avenues for understanding how DNA topology interfaces with genome function.



P177 - 177

Tau protein as a regulator of mitochondrial function and dynamics

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While pathogenic Tau mutations are known to impair mitochondrial dynamics and function, the physiological role of wild-type Tau in the maintenance of mitochondrial homeostasis remains poorly understood. Here, using the nematode *Caenorhabditis elegans* lacking PTL-1, the Tau-like homolog, and Tau knockout mice, we demonstrate that Tau deficiency enhances mitochondrial respiration, ATP production and mitophagy, promoting a pro-fusion mitochondrial state in neurons. Quantitative proteomic analyses further reveal remodeling of mitochondrial pathways in Tau deficient models. Notably, Tau mutant nematodes exhibit improved motility and increased stress resistance during aging, mediated by FZO-1/Mitofusin-dependent mitochondrial fusion. Paradoxically, loss of Tau accelerates organismal aging under basal conditions, despite enhancing mitochondrial bioenergetics and stress resilience, suggesting a context-dependent role for Tau in neuronal homeostasis. Taken together, these findings highlight a conserved function of wild-type Tau in restraining mitochondrial fusion and activity via mitofusins, thereby regulating mitochondrial quality control and energy metabolism.



P178 - 259

Investigating the role of AUP-1 in proteostasis, lipid metabolism and stress response

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The endoplasmic reticulum (ER) is central to both protein quality control and lipid droplet (LD) regulation, yet the molecular mechanisms linking these processes remain poorly understood. Ancient Ubiquitous Protein 1 (AUP1) is an ER-associated protein that also localizes to the phospholipid monolayer of LDs, suggesting a potential role in coordinating proteostasis and lipid metabolism. Despite evolutionary conservation across multicellular organisms, its precise function has remained elusive. In *Caenorhabditis elegans*, the AUP1 ortholog F44B9.5/AUP-1 is ubiquitously expressed throughout development and aging, exhibiting both diffused and punctate localization pattern reminiscent of LDs. Loss of *aup-1* results in shortened lifespan and impaired proteostasis, as evidenced by reduced proteasomal function and increased protein aggregation in nematode models of age-related proteinopathies. Moreover, AUP-1 deficient nematodes display heightened sensitivity to heat shock, glucose, and cold stress, potentially linked to lipid composition changes or proteostasis disruptions. Proteomic analysis reveals dysregulation of proteostasis-associated pathways, marked by a significant downregulation of ubiquitination and proteasomal degradation components, alongside an upregulation of heat shock response and protein folding factors. These findings suggest that AUP-1 plays a multifunctional role at the intersection of proteostasis, lipid metabolism, and stress adaptation. However, further research is needed to elucidate its precise mechanistic contributions to cellular homeostasis.



P179 - 282

Investigation of primary cilia development and structure in precancerous and drug-resistant breast epithelial cells

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Primary cilia (PC) are immotile, microtubule-based organelles that protrude from the cell surface of many eukaryotic cell types. Each PC originates from the basal body (derived from the mother centriole), which acts as a nucleation site for the ciliary axoneme (consisting of nine parallel microtubule doublets), and is surrounded by a ciliary membrane. PC dysfunctions are a major cause of numerous human developmental disorders, termed as ciliopathies, and other diseases, including cancer. Analysis of various human cancers has shown a negative correlation between tumor aggressiveness and PC presence. However, this research field remains relatively new and requires further investigation.

Primary cilia play a complex role in cancer drug resistance, functioning as a "signaling hub" that can both promote or suppress chemoresistance. Excessive primary ciliogenesis, resulting from overactivation of ciliogenesis pathways, has been shown to promote drug resistance development. Both intrinsic and acquired resistance increase both the length of primary cilia and the proportion of cells bearing primary cilia. Specific inhibition of primary ciliogenesis pathways could potentially serve as a therapeutic strategy to overcome drug resistance.

In this study, we investigated the ciliary localization of centrosome-associated proteins and their known interactors. We also examined the role of the centrosomal kinase Aurora A in primary cilia structure and development in both immortalized breast epithelial cells and a highly metastatic taxol-resistant breast cancer cell line. Our findings will provide further insight into the mechanisms of primary cilia formation and their role in cancer progression.



P180 - 291

Metabolic adaptation of lung cancer cells in response to matrix stiffness

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Lung cancer marked by a dense, fibrotic extracellular matrix (ECM) is notoriously difficult to treat. Interactions between ECM and cancer cells engage energy-intensive actin cytoskeleton remodeling and actomyosin contractility, transmitting forces that trigger signaling pathways governing cell growth and behavior. While ECM stiffness has been shown to influence metabolism in non-transformed cells, the link between cell mechanics and cancer metabolism remains poorly defined and furthering our understanding may yield novel therapeutic insights.

Here, we examine how matrix stiffness (i) alters the EGFR-driven lung cancer cell metabolism and (ii) regulates the metabolism of cells tolerant to the EGFR inhibitor Osimertinib. We used EGFR-mutant PC9 cells cultured on soft (0.5 kPa, mimicking normal lung) or stiff (25 kPa, mimicking fibrotic lung) hydrogels, and conducted transcriptomics and GC-MS based metabolomics to identify stiffness-sensitive physiological processes. Integration of both omics showed that growth on stiff vs soft matrix is characterized by higher glycolysis and cholesterol biosynthesis. Metabolomics supported increase in glutamine metabolism for glutamate synthesis (glutamylation) along with increase in serine and glycine. Interestingly, according to the transcriptomic data, treatment with Osimertinib reduced cholesterol metabolism in both soft and stiff matrices, while it inhibited glycolysis only on soft matrix.

In parallel, we generated cell lines expressing biosensors to monitor real-time ATP/ADP ratio (PercevalHR), glucose levels (FLII12Pglu-700uΔ6), and cytosolic NADH/NAD^+ redox state (Peredox-mCherry). Live-cell imaging of ATP/ADP ratio in PC9-Perceval cells revealed substantial metabolic heterogeneity across the population, with no significant ATP/ADP differences between soft and stiff substrates. Inhibition of glycolysis with iodoacetate (IAA) diminished intracellular energy by ~70% under both conditions, suggesting primarily glycolytic dependency regardless of ECM stiffness. Investigations of Osimertinib effect on energy status and how stiffness and drug treatment interact to shape metabolic state are ongoing.

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P181 - 28

Characterization of the novel A30G α-Synuclein mutant in Parkinson's Disease

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Our main aim is to unravel the biochemical properties of the novel A30G α-synuclein (α-syn) mutant in a cellular context, deciphering the mechanisms governing aggregation and induced toxicity, leading to a better understanding of Parkinson's Disease pathogenesis. Hence, we constructed stable neuroblastoma cell lines overexpressing WT or A30G α-syn and assessed the proteomic profile, the α-syn-induced toxicity, as well as the seeding and aggregation propensity of the A30G α-syn compared to WT. In the proteomic analysis, we observed differences regarding several metabolic pathways involved in neurodegenerative disease pathology, i.e. cellular cell response, membrane organization, lysosome and mitochondria homeostasis. To verify further, we assessed A30G α-Syn-induced toxicity by measuring ROS levels, caspase activity and cell viability. A30G cells displayed an early pattern of oxidative and mitochondrial stress, decreased TH levels, followed by increased cell death. Additionally, we assessed protein levels and the formation of α-syn aggregated species. It seems that A30G cells form mainly soluble α-syn species with limited phosphorylation, compared to WT cells. Using WT and A30G α-syn recombinant fibrils (PFFs) in WT and A30G differentiated neuroblastoma cells, as well as in iPSC-derived dopaminergic neurons, we examined seeding and aggregation of endogenous α-syn, as well as the induced cell stress. Upon PFF addition, seeding and aggregation of endogenous α-syn occurred within 4 days of PFF incubation, resulting in the accumulation of insoluble α-syn species, that were further increased when A30G PFFs were used in both cell models. A30G PFFs induced ROS production in both neuroblastoma cell lines, while A30G cells exhibited increased oxidative stress regardless of the PFF strain. Our results highlight that A30G α-syn seems to be less potent to form aggregates that are less phosphorylated than WT α-syn, nonetheless is efficiently inducing cellular stress and cell death, propagating α-syn-induced pathology.



P182 - 77

The defects of macrophage antibacterial immune response in aging and DNA damage accumulation

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Aging is a complex process associated with accumulation of DNA damage, leading to a gradual decline of immune cell function, known as immunosenescence, and a dysregulated inflammatory response, known as inflammaging. These characteristics contribute to increased likelihood for development of chronic and acute comorbidities, including increased susceptibility to infections. Macrophages constitute the first line of defense against infectious agents, as they recognize and uptake pathogens, facilitating their rapid clearance. However, their effectiveness against pathogens in aging and due to age-related DNA damage accumulation remains elusive.

To address this, we utilized Bone Marrow Derived Macrophages (BMDMs) from young adult and aged mice as well as from Ercc1$^{F/+}$ or Ercc1$^{F/-}$ mice, that have defective DNA damage response mechanism and accumulate DNA damage in macrophages. Cells were stimulated with TLR2 or TLR4 ligands and transcriptomic profile as well as expression of cytokines were assessed. We further analyzed the protein levels of the histone demethylases PHF8 and PHF2, critical regulators of macrophage responses. Finally, we exposed macrophages to Group Beta Streptococcus (GBS) and evaluated their phagocytotic capacity, the intracellular bacterial load and their ability to activate antibacterial autophagy. The results showed that aged mice exhibited enhanced responses to TLR ligands, as determined by elevated production of inflammatory cytokines, and higher PHF2 and PHF8 protein levels, whilst Ercc1$^{F/-}$ mice display the opposite phenotype. Additionally, both macrophages from aged mice and Ercc1$^{F/-}$ mice displayed defective induction of autophagy, but they presented substantial differences in phagocytic capacity and intracellular GBS load.

Our findings demonstrate that aging and DNA damage accumulation elicit distinct epigenetic, inflammatory and antibacterial responses in macrophages. Thus, unraveling the complexity of these mechanisms will help us understand the contribution of macrophages in the susceptibility of aged individuals to infections.

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Intracellular calcium regulation in erythrocytes directly influences their resilience to corpuscular and redox stress

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Intracellular calcium in mature erythrocytes has multiple roles. It maintains erythrocyte volume, membrane integrity, and physiological functions, including redox balance, but it also mediates cellular aging. Elevated intracellular calcium concentrations can, thus, lead to pathological conditions. Moreover, calcium responds dynamically to erythrocyte stress, a phenomenon that we aimed to address in this study. Variations in intracellular calcium were monitored to assess their influence on erythrocyte hemolytic and oxidative profile. Erythrocytes from thirty healthy donors were collected and subjected to biochemical assays and proteasomal activity assessment, since the proteasome complex constitutes the major homeostasis mechanism of erythrocytes. Afterwards, the samples were subdivided into two groups, based on their intracellular calcium levels: higher and lower. From a hemolytic perspective, the two groups did not differ in innate hemolysis. However, the low-Ca^{2+} group showed reduced resilience under proteotoxic hemolysis, induced by a pan-proteasome inhibitor, and exhibited higher osmotic fragility (113.43±23.89 vs 60.04±36.17, $p<0.01$; 0.43±0.03 vs 0.40±0.02, $p<0.012$). The same group also exhibited lower intracellular ROS levels (1559±386 vs 1931±446) and greater resistance to general oxidants such as tert-butyl hydroperoxide and to hemoglobin-specific oxidation via phenylhydrazine (2060±281 vs 3180±1205; 31048±4393 vs 40914±9959, $p<0.01$). Membrane-associated proteasomes showed higher chymotrypsin- and trypsin-like activities, while cytosolic ones demonstrated trends for lower proteolytic activity. The low-Ca^{2+} group exhibited fewer membrane-bound hemichromes and lower externalized phosphatidylserine, both markers of aging (3.19±1.55 vs 4.51±1.39; 0.57±1.46 vs 0.87±0.46). These profiles suggest proteasome translocation toward the membrane in low-Ca^{2+} cells, enhancing oxidized hemoglobin clearance and oxidative stress management. Conversely, high-Ca^{2+} erythrocytes were more prone to hemolysis, likely due to limited Gardos channel activation, leading to stiffer membranes and fragility. Overall, these findings highlight the regulatory role of intracellular calcium in balancing erythrocyte stability among healthy donors. Modulation of intracellular calcium may be explored to optimize erythrocyte resilience under iatrogenic or pathological conditions.



P184 - 119

Association of steroid hormone receptors signalling with skeletal muscle pathophysiology and aging

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Aging is driven by various endogenous and exogenous stressors and is characterized by a gradual decline in all physiological functions, a decrease in repair mechanisms, senescence and eventually death [1]. Aging leads to significant alterations in the endocrine system, but imbalances in the endocrine system also affect the aging process [2]. Therefore, there is increasing interest in understanding the association between aging and endocrine function. In this study, we focus on understanding the interplay between skeletal muscles aging and steroid receptors signaling. Emphasis was given to glucocorticoids hormones that constitute the primary stress hormones [3]. There are several models of induced cellular senescence. D-galactose-induced senescence is a prominent model for studying accelerated aging by causing oxidative stress, mitochondria dysfunction and inflammation [4]. Chronic kidney disease is also characterized by oxidative stress, mitochondrial dysfunction, inflammation, and uremia that can lead to uremic myopathy and muscle atrophy [5]. Therefore, in this study D-galactose induced aging in C2C12 cells and gastrocnemius muscle of uremic rabbit with chronic kidney disease was used as an *in vitro* and *in vivo* model, respectively, to study the association of steroid receptors signaling with muscle aging and uremic- induced myopathy. MTT assay, Western blot and immunofluorescence analysis were applied for that purpose. Our results revealed alteration of GR protein levels and modulation of its subcellular localization that were accompanied by changes in protein levels of molecules related to GR signalling such as PEPCK, PPARα, OXPHOs subunits, as well as changes in protein levels of molecules related to protein transport, mitochondrial biogenesis and apoptosis. Our results further support the close association of GR signalling with aging, the exact mechanism of which remains to be elucidated.

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In vitro and *in vivo* assessment of rabbit adipose-derived mesenchymal stem cells' regenerative potential for the treatment of intervertebral disc degeneration; the role of cellular senescence

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Implantation of mesenchymal stem cells (MSCs) appears to be a promising choice for intervertebral disc (IVD) regeneration. Among MSCs, adipose-derived MSCs (ADSCs) have shown stronger differentiation ability towards a nucleus pulposus (NP)-like phenotype. The aim of the current study was to assess the IVD regenerative potential of rabbit ADSCs (rADSCs) *in vitro* and *in vivo*. Given that preceding propagation by serial *in vitro* subculturing is often required to achieve high numbers of viable cells necessary for tissue regeneration applications (which could result in senescence induction), we explored the effect of senescence on rADCSs' molecular profile and function *in vitro*, as well as its direct effect on the final outcome of IVD tissue quality for the first time, when injected intradiscally in a rabbit model. Young rADSCs were shown to possess a high chondrogenic differentiation potential and were found to generally respond in a similar way with NP IVD cells to high osmolality *in vitro*, both traits being beneficial for their subsequent use in IVD regenerative applications. On the other hand, senescent rADSCs displayed a complete loss of their chondrogenic potential and a highly catabolic molecular profile *in vitro* (up-regulation of MMP1, MMP3 and MMP13 and down-regulation of the proteoglycan aggrecan), which foreboded a negative impact on IVD tissue quality if used in a cell-based therapy. Indeed, delivery of young rADSCs into the NP of rabbit IVDs *in vivo* resulted in higher staining intensities of collagen type II and aggrecan, in contrast to senescent rADSCs, which were not only ineffective, but they could also worsen IVD histological features. Our results indicate that in-depth characterization to timely track and exclude senescent ADSCs prior to transplantation should be included as part of the quality control in ADSC-based therapies against IVD degeneration, to warrant the utmost regenerative effect.

Reference:

Mavrogonatou E.[§], Korompilia A-M.[§] et al., The Spine Journal. 2025 (under revision).
([§]Authors contributed equally to this work)



P186 - 298

Distinct organismal and tissue outcomes after Aβ-42-induced proteotoxic stress in *Drosophila melanogaster* neural cells

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Age-associated neurological diseases are among the leading causes of illness and disability worldwide. Brain aging and many neurodegenerative diseases are characterized by the progressive accumulation of protein aggregates that affect proteome functionality, which along with the gradual age-related decline of cellular stress responses, leads to tissue homeostasis deterioration. Research studies on brain aging have so far focused mainly on neuronal cells; however, evidence indicates that glial cells are the primarily cellular type that respond and undergo structural and functional changes during aging. *Drosophila melanogaster* is a valuable model organism for brain aging studies, with neuronal tissue consisting of both neurons and three distinct glial subtypes. In the present study, we aimed to characterize alterations at the tissue and organismal level, following the cell-specific proteotoxic stress mediated by the overexpression of the human amyloid beta 1–42 (Aβ-42) peptide. We focused on the main proteolytic pathways of the Proteostasis Network (PN), namely the ubiquitin-proteasome (UPP) and autophagy-lysosome (ALP) pathways, as well as the antioxidant response NRF2/KEAP1 pathway. Our data showed that the Aβ-42 overexpression in neurons caused a toxic phenotype with a sharp decline in lifespan and locomotion of flies, accompanied by reduced antioxidant response and proteostatic adaptations. Interestingly, the same genetic intervention in glial population led to a milder reduction in flies life expectancy and climbing activity, while distinct glial subtypes exhibited different proteostatic adaptations. Our findings highlight the differential responses of neural cell subtypes to proteotoxic stress, revealing their diverse impact on brain aging and the potential for cell-targeted strategies in the prevention or therapy of brain diseases.

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Cell-specific activation of the antioxidant transcription factor NRF2 reveals systemic proteostatic and metabolic crosstalk between the brain and gut in *Drosophila melanogaster*

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The Proteostasis Network (PN), the cellular system that maintains proteome quality, is dynamically regulated across cell types and tissues to ensure organismal health and longevity. Its function relies on coordinated signaling pathways operating at the cellular, tissue, and systemic levels. Deterioration of tissue-specific protein quality control mechanisms can trigger compensatory stress responses in other tissues, emphasizing the systemic nature of the proteostatic network. The Central Nervous System (CNS) is known to influence intestinal physiology under stress, while recent research has revealed bidirectional communication between the brain and gut, collectively known as the gut-brain axis (GBA). The GBA not only maintains gastrointestinal homeostasis but also contributes to higher CNS functions. However, the molecular mechanisms governing this inter-tissue communication remain poorly understood. *Drosophila melanogaster* provides an excellent model for studying these processes due to the high similarity of its brain and intestinal structures to those of mammals. In this study, we aimed to investigate, at a systemic level, the effects of cell- or tissue- specific overexpression of the antioxidant response transcription factor cncC (the *Drosophila* homologue of mammalian NRF2). To this end, we genetically induced NRF2/cncC expression in different cellular populations -neurons, glial cells and gut subtypes including intestinal stem cells, enteroendocrine cells, and enterocytes- and examined the resulting alterations in antioxidant, proteostatic, mitostatic, and metabolic pathways within both brain and gut tissues. Our results demonstrate that NRF2/cncC activation exerts distinct effects depending on the targeted tissue, influencing not only local but also distant tissues. These findings highlight the systemic impact of tissue-specific stress responses and underscore the importance of inter-tissue communication mechanisms in maintaining organismal homeostasis.

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Expression, Purification and Functional study of the RING and RING-DTC Domains of the Human E3 Ubiquitin Ligase DTX3L

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Human Deltex (DTX) proteins are RING-type E3 ubiquitin ligases that modulate key cellular signalling pathways. The five human DTX family members share a conserved C-terminal Deltex (DTC) domain and a RING domain but differ in their N-terminal regions. Among them, human DTX3L is unique, as it exhibits distinct structural and functional features and has been implicated in cancer progression, DNA damage repair, and interferon-mediated antiviral responses. DTX3L contains an N-terminal region predicted to include an RNA Recognition Motif (RRM) and five K Homology (KH) domains, in addition to the conserved RING-DTC (RD) region. The KH domains mediate interactions with intracellular partners such as hPARP9 and hPARP14, forming a multimeric complex involved in cellular signalling and immune regulation[1]. The C-terminal RING domain recruits the E2-ubiquitin conjugate and promotes formation of the closed E2-ubiquitin conformation, while the adjacent DTC domain binds ADP-ribose (ADPr) and ADP-ribosylated substrates. A flexible linker connecting the two domains is proposed to position the E2-ubiquitin complex close to its substrate, facilitating ubiquitin transfer[2]. This study will characterize the molecular mechanisms underlying DTX3L function, driven by RING and RING-DTC domains. Specifically, it investigates the interaction between DTX3L and the E2 enzyme UbcH5b and explores potential viral substrates targeted by DTX3L-mediated ubiquitination. Recombinant expression and purification of DTX3L and UbcH5b will be followed by biophysical analyses, including conformational dynamics by NMR, thermodynamic characterization by ITC to quantify binding affinities, and biochemical ubiquitination assays to assess the ubiquitination capacity of DTX3L on viral proteins.
Overall, this work aims to elucidate the way that DTX3L cooperates with UbcH5b to mediate substrate ubiquitination and how this mechanism contributes to cellular antiviral defense and infection regulation.

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P189 - 10

Investigation of antiplatelet properties of dietary supplements

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Dietary supplements derived from tomato extract and red yeast rice have been associated with a reduced risk of atherothrombotic events. The aim of the present study was to investigate the inhibitory effect of tomato extract and red yeast rice on platelet aggregation, as well as to evaluate potential synergistic or additive actions between them. The effect of tomato extract at concentrations of 10–90 μg/mL and of red yeast rice up to 40 μg/mL on platelet aggregation induced by TRAP-6, arachidonic acid (AA), ADP, and collagen was studied. For this purpose, platelet-rich plasma (PRP) was isolated from healthy volunteers, and the response was recorded after the addition of the supplements. Tomato extract exhibited a strong inhibitory effect against aggregation induced by TRAP-6 (maximum inhibition at 30 μg/mL), AA (threshold of activity at 20 μg/mL), and ADP (maximum inhibition approximately 40%). In the case of collagen, the inhibition was lower (30%) and required much higher concentrations (90 μg/mL). Red yeast rice, even at its maximum concentration, did not show a significant inhibitory effect against AA, while it displayed limited activity against the other agonists, as at 40 μg/mL inhibition reached 30% with TRAP-6, 20% with collagen, and 5% with ADP. The combination of the two substances at low concentrations did not show synergy, whereas at other concentration combinations a strong synergistic effect was observed (91% inhibition). Moreover, at levels corresponding to the recommended daily doses (30 and 40 μg/mL for tomato and rice, respectively), an additive effect was observed against ADP-induced aggregation (45% inhibition). Tomato extract constitutes a significant inhibitor of platelet aggregation, particularly against TRAP-6 and AA, while red yeast rice exhibits more limited efficacy. Their combination may enhance the inhibitory effect; however, the observed synergy depends on the concentrations used. Further studies are required to elucidate the underlying mechanisms.



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Identification of cathepsin S cysteine protease as a novel processor of extracellular α-synuclein

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Alpha-synuclein (AS) aggregates in the brain are a hallmark of Parkinson's disease (PD). Very recently, we showed that extracellular vesicles (EVs) isolated from mouse brain carry active proteolytic enzymes that can cleave the monomeric AS and AS fibrils [1]. Interestingly, cathepsin S (CTSS) was identified in EVs, where it is active, as demonstrated by enzyme kinetics using the LHVS specific inhibitor of CTSS [1]. Other proteases (*e.g.*, CTSB and CTSD) are known to cleave AS. We showed that proteolytic processing of AS by KLK6 protease can alter the uptake of AS by neuronal cells [2] and alleviates PD pathology in a mouse model [3]. Here, we investigated whether CTSS can recognize AS as specific substrate, and whether proteolysis of AS by CTSS could affect AS fibrillation. Indeed, we show that CTSS can efficiently cleave the monomeric human (and mouse) AS *in vitro*, originally yielding distinct proteolytic fragments, while it can fully degrade AS in 30 min. The peptide bonds in AS that are cleaved by CTSS and the cleavage sequence were determined by LC/MS/MS. It was found that 4 of 5 scissile bonds are located in the NAC region of AS, which is responsible for its aggregation propensity. Further, we studied whether the AS fragments produced by limited proteolysis of AS by CTSS could affect the fibrillation of monomeric AS. Using these fragments to template AS fibrillation, we found no effect on the AS seeding capacity. Nonetheless, CTSS can cleave AS fibrils quite efficiently, therefore, its activity in the brain could be implicated in spreading of the disease. The uptake of AS fibrils templated by CTSS-processed AS was studied with SH-SY5Y cells. For the first time, we provide evidence that CTSS can process AS monomer and fibrils in the extracellular space, and that CTSS could be implicated in PD pathology.

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The GPCR lipid/androgen receptor OXER1 regulates neutrophil migration and cytotoxicity in the tumor microenvironment

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Background: The oxoeicosanoid receptor 1 (OXER1) is a G-protein-coupled receptor activated by 5-oxo-eicosatetraenoic acid (5-oxo-ETE), a lipid mediator derived from the 5-lipoxygenase pathway and known chemoattractant for leukocytes. Additionally, OXER1 plays a role as a membrane androgen receptor with androgens antagonizing 5-oxo-ETE actions. While OXER1 has been implicated in inflammation, its role in modulating neutrophil behaviour in the tumor microenvironment remains unclear. In this study we aimed to investigate how 5-oxo-ETE/OXER1 regulates neutrophil migration, tumor-neutrophil interactions, and neutrophil-mediated cytotoxicity against cancer cells.

Materials and methods: Differentiated HL-60 (dHL-60) cells were used as a neutrophil model. Cell migration was analyzed using scratch, transwell and 3D spheroid co-culture assays for prostate and breast cancer cells (DU-145 and T47D respectively). OXER1 activity was antagonized by Gue 1654 and testosterone-BSA, while Zileuton was used to inhibit 5-oxo-ETE synthesis by 5-LOX. Cancer cell migration and viability were assessed following exposure to neutrophil-conditioned media or direct co-culture.

Results: 5-oxo-ETE/OXER1 activation significantly enhanced neutrophil migration, whereas OXER1 antagonists abolished this effect. Co-culture with cancer cells increased neutrophil migration via tumor-derived soluble factors, an effect blocked by OXER1 or 5-LOX inhibition. In spheroid assays, OXER1 blockade delayed neutrophil infiltration toward tumor spheroids. Conditioned medium from neutrophils enhanced the migratory capacity of both prostate and breast cancer cells through OXER1, while inhibition of OXER1 or 5-oxo-ETE synthesis reversed this effect. Notably, OXER1 inhibition augmented neutrophil-mediated cytotoxicity against tumor cells in co-culture, an effect requiring direct cell-cell contact and reversed by exogenous 5-oxo-ETE.

Conclusion: These findings identify OXER1 as a critical regulator of neutrophil function in cancer. The 5-oxo-ETE/OXER1 axis promotes reciprocal communication between neutrophils and tumor cells, driving mutual migration and supporting tumor progression. Conversely, OXER1 inhibition shifts neutrophils from a pro-migratory to a cytotoxic, antitumor phenotype, highlighting OXER1 as a promising therapeutic target for modulating tumor-associated neutrophil responses.



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Assessment of curcumin-conjugated PEGylated magnetic nanoparticles in biomedical applications

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PEGylated magnetic nanoparticles are versatile drug delivery/theranostic platforms, the bioactivity of which depends on the core phase and synthetic route. These PEG-MNPs were used as carriers for curcumin (Cur), a pleiotropic molecule with antitumor and antioxidant activities[1–4]. Six formulations were synthesized: PEG-Fe_3O_4 via two-pot co-precipitation, PEG-Fe_3O_4-Cur, its Cur-loaded analogue; Fe_3O_4-Cur, one-pot Fe_3O_4 co-precipitated with Cur; Fe_3O_4-Cur-PEG, its post-PEGylated derivative; PEG-α-Fe_2O_3, hydrothermal; and PEG-α-Fe_2O_3-Cur, its Cur-loaded counterpart.

Murine neuroblastoma (N2a) and neuroglioma (GL261) cells were exposed to 0.244–250 μg/mL of each formulation. Cell viability was determined by the MTT assay (at 24h) and superoxide anion ($O_2^{\bullet-}$) levels were determined by the NBT assay (at 3 and 6h). One-way ANOVA/Dunnett's test was applied for statistical analysis.

PEG-Fe_3O_4 at low-doses (≤31.25 μg/mL) decreased mortality in N2a cells but was cytotoxic at ≥31.25 μg/mL. Cur loading abolished this hormesis. PEG-α-Fe_2O_3 reduced viability at 0.244 μg/mL and at higher doses, indicating a core-phase effect (α-Fe_2O_3>Fe_3O_4). Low concentrations of Fe_3O_4-Cur moderately increased N2a viability but high concentrations (31.25 μg/mL) were cytotoxic. Its post-PEGylation rendered the particle highly cytotoxic.

GL261 cells were more sensitive: most formulations reduced viability at concentrations as low as 0.244 μg/mL and at higher concentrations the viability values did not decrease in a strictly concentration-dependent manner across all tested doses. PEG-α-Fe_2O_3 and PEG-α-Fe_2O_3-Cur were most potent PEG-Fe_3O_4 and PEG-Fe_3O_4-Cur, were intermediate. Cur reduced viability in both cell-types and was overtly toxic at high concentrations. Transient superoxide elevations at 3h, gradually reducing at 6h, indicate ROS-mediated cell-death.

Collectively, the synthetic route and the sequence in which Cur and PEG are introduced dictate the cytotoxic profile of the nanoparticles in a cell-line-specific manner.All nanoformulations were cytotoxic at ≥31.25 μg/mL. These experiments establish a baseline for future studies to determine IC50/IC20 concentrations, their potential anti-tumorigenic effects, and any potential impact on healthy cells.

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P193 - 64

Evaluation of antibodies for detection of LEKTI, the causative factor of Netherton syndrome, using CRISPR/CAS9

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Netherton syndrome (NS) is a rare epidermal genetic disease due to inactivating mutations in *SPINK5* gene encoding LEKTI, a multidomain protease inhibitor of 1064 residues [1]. This leads to unopposed activities of kallikrein (KLK) proteases in the stratum corneum-stratum granulosum interface which causes epidermal overdesquamation, constitutive inflammation, and allergies [2]. NS shares common underlying mechanisms with psoriasis and atopic dermatitis. Thus, study of NS pathophysiology provides valuable insights into fighting common diseases. Several NS studies have used homemade anti-LEKTI antibodies in Western blotting (WB) [3,4] causing inherent issues in cross-validating experimental results from different laboratories. On the other hand, commercial anti-LEKTI antibodies available from various suppliers have not been validated. The International Working Group for Antibody Validation (IWGAV), an *ad hoc* committee of scientists from diverse disciplines, has highlighted the need for antibody validation [5] and suggested for antibody validation the genetic strategy that involves targeted inactivation of the gene encoding the protein recognized by the tested antibody [5]. Here, we validated, using WB, the specificity of four commercial anti-LEKTI antibodies in biological samples. All tested antibodies recognize different epitopes present at the *N*-terminus, in the middle or at the *C*-terminus of LEKTI. In compliance with IWGAV, we have generated human *SPINK5*$^{-/-}$ keratinocytes by CRISPR/CAS9, confirmed the complete ablation of *SPINK5*, and compared LEKTI protein levels in *SPINK5*$^{+/+}$ and *SPINK5*$^{-/-}$ keratinocytes under naïve and differentiating stages. We found that none of these antibodies could specifically detect LEKTI, and all yielded patterns consisting of multiple bands in both *SPINK5*$^{-/-}$ and *SPINK5*$^{+/+}$, apparently a result of antibody crossreactivity with unrelated proteins, which can lead to false conclusions in immunoassays developed to detect LEKTI. These findings highlight the need to develop and validate new specific anti-LEKTI antibodies for diagnostic and research applications, in order to provide robust results.

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Study of variants of uncertain clinical significance in homologous recombination genes

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DNA damage caused by homologous recombination (HR) deficiency has been linked to certain types of neoplasms. A PARP inhibitor (PARPi) can effectively target tumors with HR deficit caused by germline or somatic mutations. In cancer patients, genetic analysis of HR genes can reveal variants of uncertain clinical significance (VUS). Further study of these variants is required to reclassify them, unravel gene function, and provide new insights into structure-function relationships.

In this context, we studied the pathogenicity prediction of VUS in key HR genes, such as BRCA1 and BRCA2, using AI-based tools (eg AlphaMissense), and compared predictions to functional studies that screen many random mutations in crucial protein domains. This analysis validated Alphamissense as an accurate predictor of variant pathogenicity and allowed the analysis of variants identified in the Greek population.

Many genes involved in the HR pathway remain poorly studied, amongst them FANCD2, which was recently shown to play a crucial role in replication fork protection. Bioinformatic analysis revealed ~600 missense VUS observed in patients. Using Alphamissense, the majority are classified as benign/likely benign, ~60 variants as pathogenic, whereas 45 remain ambiguous. For predicting patient response to PARPi, a direct assay of PARPi sensitivity conferred by variants in HR genes is required. To that end, we set up clonogenic assays using U2OS cells bearing a knock-out of FANCD2 and showed increased sensitivity upon PARPi administration. This assay can thus be used for disease modeling, by introducing selected FANCD2 variants from patients, to study their effect on PARPi sensitivity and analyse molecular pathways.

Through this study, we aim to reclassify FANCD2 VUS according to their PARPi sensitivity, enabling a more refined characterization of the gene and its role in HR, as well as supporting informed clinical management and genetic counseling.



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Generation of a three-dimensional reconstructed skin model for Netherton syndrome

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Netherton syndrome (NS) is a rare (1/200,000 births) genodermatosis due to inactivating mutations in *SPINK5* gene encoding LEKTI, a multidomain protease inhibitor [1]. LEKTI deficiency leads to unopposed proteolytic activities in the stratum corneum-stratum granulosum interface, mainly conferred by KLK5, KLK7, and KLK14. This highly elevated proteolysis is causally linked to pathological skin overdesquamation, constitutive inflammation, and allergies [2]. NS shares common molecular mechanisms with psoriasis and atopic dermatitis. Thus, study of NS pathophysiology provides valuable insights to fight common diseases. *Spink5*$^{-/-}$ mice recapitulate NS symptoms but die uniformly within 5 hours from birth due to severe dehydration caused by the severe defect of the epidermal barrier [3-5], consequently, this model cannot be used to test potential therapeutic strategies. Neonatal lethality of *Spink5*$^{-/-}$ combined with the tendency to reduce the numbers of experimental animals (compliance with 3R principle) makes it necessary to seek alternative models. Here, we have generated *SPINK5*$^{-/-}$ keratinocytes using CRISPR/CAS9 technology with the aim to develop an organotypic skin model of human NS. Three targeting strategies were applied: deletion of exon 1, deletion of exons 1-3, and deletion within exon 15 (middle of *SPINK5*). Deletions were verified by genomic PCR and DNA sequencing. *SPINK5*$^{-/-}$ keratinocytes were grown on the top of a collagen gel containing human fibroblasts to mimic the dermis. Differentiation was induced by exposing the keratinocyte layer to the atmosphere. The organotypic skin was analyzed by histology and immunofluorescence to assess its micro-architecture and the expression of key differentiation proteins in comparison to human skin biopsies. Epidermal proteolytic activities were quantified by *in situ* zymography and found elevated in the absence of *SPINK5*, reminiscent of NS patient skin. Collectively, the generated organotypic model displays key characteristics of NS and it can be used for testing potential new therapeutics but also to identify new pathophysiological mechanisms.

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P196 - 126

Exploring the role of macrophage-derived Versican in pulmonary fibrosis

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Pulmonary fibrosis is a progressive and often fatal lung disease characterized by excessive extracellular matrix (ECM) deposition and tissue remodeling driven by activated fibroblasts. Proteoglycans (PGs) are a significant component of the interstitial ECM, which fine-tune the overall tissue architecture determined by fibrous proteins, such as collagens, and regulate several ECM-controlled signaling pathways. Versican (VCAN), produced predominantly by fibroblasts and representing the most abundant PG in fibrotic lungs, was recently shown by our group to act as an autologous "fibrotic brake", controlling the underlying ECM composition, structure and mechanotransduction, and suppressing fibroblast invasion and pulmonary fibrosis. In contrast, emerging data indicate that VCAN expression by macrophages (MΦs) may play an opposing, pro-fibrotic role. To dissect the compartment-specific contribution of VCAN to pulmonary fibrosis, we generated chimeric mice with *Vcan* haploinsufficiency confined to either stromal or myeloid compartments via bone marrow transplantation. Consistent with previous findings, *Vcan* haploinsufficiency in stromal cells exacerbated bleomycin (BLM)-induced pulmonary fibrosis. Strikingly, myeloid-specific *Vcan* haploinsufficiency attenuated fibrosis severity, suggesting that MΦ-derived VCAN promotes fibrotic pathology. To confirm this, we utilized LysM-Cre–driven deletion of *Vcan* in myeloid cells. MΦ-specific *Vcan* knockout mice exhibited reduced BLM-induced pulmonary fibrosis, as assessed by histopathology, lung mechanics, and gene expression analyses. Complementary *in vitro* experiments using bone marrow–derived macrophages (BMDMs) revealed that *Vcan* deletion dampened inflammatory signaling and cytokine production upon LPS stimulation, coinciding with decreased phosphorylation of key MAPK pathway components. Collectively, these findings identify macrophage-derived VCAN as a novel pro-inflammatory and pro-fibrotic mediator in pulmonary fibrosis, in contrast to the anti-fibrotic role of fibroblast-derived VCAN. Understanding these cell-specific functions of VCAN may inform the development of targeted therapeutic strategies aimed at modulating ECM–immune cell interactions in fibrotic lung disease.



P197 - 140

Investigating the localized LL-37 elevation and neutrophil activity in RSV-positive patients

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Respiratory Syncytial Virus (RSV) remains a major health concern, since it constitutes the leading cause of hospitalization among infants in developed countries and second most frequent reason of lower respiratory mortality globally. Neutrophils play a significant role in the host innate immune response and serve as the body's first line of defense against infection. One of the three strategies they utilize in the elimination of invading microbes is the release of Neutrophil Extracellular Traps (NETs) which are composed of decondensed chromatin, decorated with antimicrobial proteins. Previous studies have shown that the human cathelicidin, also known as LL-37, in its active form, induces release of NETs with antimicrobial activity. Our group collected clinical samples (serum and nasopharyngeal) from patients who tested positive for RSV and evaluated their ability to provoke NETs release, as well as the levels of LL-37. Nasopharyngeal samples exhibited higher LL-37 levels, while LL-37 was almost absent in the respective serum ones, indicating that the antimicrobial peptide accumulates and acts on the site of infection.



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Exploring the functional role of SARS-CoV-2 ORF9b in epithelial and immune cells

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SARS-CoV-2 encodes several accessory proteins that, although dispensable for viral replication, play crucial roles in modulating host cellular processes and evading immune defenses. Among them, the accessory protein ORF9b, a 97-amino acid peptide translated from an alternative open reading frame within the nucleocapsid (N) gene, has been linked to mitochondrial regulation and suppression of type I interferon signaling.

In this study, we investigated the cellular consequences of ORF9b expression in two human cell lines: alveolar epithelial A549 cells and T-lymphocyte Jurkat cells, representing epithelial and immune compartments, respectively. Stable ORF9b-expressing cells were generated via lentiviral transduction, individual cell clones were isolated, and ORF9b expression was confirmed at both transcriptional and translational levels. The generated cells were examined for changes in cell viability and proliferation (SRB and Alamar Blue assays), alterations in cell cycle progression (flow cytometry), and transcriptional modulation using RNA sequencing and real-time PCR of selected genes.

Preliminary results indicate that ORF9b expression influences cell proliferation and the transcriptional landscape of key host pathways in a cell type-dependent manner. These findings suggest that ORF9b contributes to SARS-CoV-2-mediated cellular reprogramming and may play a broader role in viral pathogenesis. Further mechanistic analysis is underway to clarify the molecular alterations underlying these effects.



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Targeting MCT1 and MCT4 in Non-Small Cell Lung Cancer (NSCLC) through in vitro and translational analyses

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Cancer cells undergo metabolic reprogramming to promote their survival. Monocarboxylate transporters MCT1 and MCT4 regulate lactate transport, supporting tumor growth and metastasis. Their overexpression has been reported in non-small cell lung cancer (NSCLC) tissues and circulating tumor cells (CTCs), correlating with patients' prognosis. This study evaluated the effects of the dual MCT1/MCT4 inhibitor, Syrosingopine, on NSCLC (H1975) and CTC-derived (CTC-MCC-41) cell lines, as well as on real CTCs isolated from NSCLC patients. Cells were treated with Syrosingopine, and cell viability was determined via MTT assay. Apoptosis was assessed by M30 immunofluorescence staining, while mitochondrial ROS (mtROS) levels were quantified using MitoSOX™ Red. Peripheral blood mononuclear cells (PBMCs) from four NSCLC patients were also cultured, treated with Syrosingopine, and stained for CK, CD45, M30, MCT1, and MCT4 antibodies. Samples were analysed using the VyCAP platform, followed by statistical analyses. An IC_{50} of 13μM Syrosingopine was established after 48 hours of treatment and used for all subsequent experiments. MCT1/MCT4 inhibition significantly reduced cell viability by 46% (p=0.0029) in H1975 and 21% (p=0.0052) in CTC-MCC-41 cells. Apoptotic (M30-positive) cells increased after Syrosingopine treatment by 370% (p=0.0004) in H1975, and 45% (p<0.0001) in CTC-MCC-41, compared to control. MitoSOX fluorescence analysis demonstrated a significant increase of 140% (p=0.0001) and 90% (p=0.0022) in mtROS levels in H1975 and CTC-MCC-41 cells, respectively, after treatment. In patients' samples, tumor cells were defined as (CK+/CD45-), and/or (CK-/CD45-) with nuclei size ≥10μm. Total (CK-/CD45-) and MCT1-positive cells were decreased (p=0.0562 and p=0.1289, respectively) after treatment. Dual MCT1/MCT4 inhibition decreases cell viability, while enhancing apoptosis and mtROS levels. These findings indicate that targeting lactate transport may disrupt tumor metabolic adaptation and metastasis. However, further experiments in NSCLC patients' CTCs are warranted to confirm the clinical relevance of these findings in metastatic procedure.



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Elevated expression of SOCE channel components in circulating tumor cells (CTCs) from breast cancer patients

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Breast cancer is the most common malignancy in women, with metastasis being the leading cause of the disease-related death. Circulating tumor cells (CTCs), are key mediators of metastatic dissemination, serve as valuable markers for early diagnosis, and personalized therapy. However, their extremely low levels in the bloodstream necessitate the identification of new biomarkers. The molecules STIM1 and ORAI1, key components of the Store-Operated Calcium Entry (SOCE) pathway involved in Ca^{2+} signaling, have been associated with cancer cell migration and metastatic potential. Therefore, they may serve as promising biomarkers for identifying CTCs in breast cancer patients.

This study aimed to evaluate the expression of STIM1 and ORAI1 in CTCs isolated from breast cancer patients and investigate their potential prognostic value. A total of 60 baseline patients were enrolled in this study (42% of them had metastasis). PBMCs were isolated with Ficoll density gradient, followed by triple immunofluorescence staining using CK (as a CTC marker), STIM1, and ORAI1 antibodies. The samples were analyzed with the VyCAP platform, followed by statistical analysis using the SPSS software. CTCs were detected in 42% of the Ficoll samples. Among these patients, 48% exhibited the (CK+/STIM1+/ORAI1+) phenotype, followed by 39% and 13% with (CK+/STIM1+/ORAI1-) and (CK+/STIM1-/ORAI1-) phenotypes, respectively. Notably, none of the CTCs showcased the (CK+/STIM1-/ORAI1+) phenotype. STIM1 was detected in 88% of the total isolated CTCs, whereas ORAI1 was identified in 48% of them. Statistical analysis revealed that metastatic patients with the (CK+/STIM1+/ORAI1-) phenotype, had significantly lower overall survival (OS: p=0.011, HR=5).

STIM1 and ORAI are overexpressed in CTCs isolated from breast cancer patients, and their expression was correlated with patients' outcomes. In particular, the (CK+/STIM1+/ORAI1-) phenotype is related to reduced OS in metastatic cases. However, additional large-scale studies are needed to validate the clinical relevance of these biomarkers.



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Expression of STIM1, ORAI1, and CD98hc in circulating tumor cells (CTCs) isolated from pancreatic cancer patients

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Pancreatic cancer (PC) remains an aggressive malignancy with a poor clinical outcome. Circulating Tumor Cells (CTCs) offer a promising, non-invasive tool for monitoring disease progression and metastatic potential. Overexpression of STIM1 and ORAI1, core regulators of calcium signaling, and CD98hc, an essential amino acid transporter, has been shown to enhance cancer cell survival and migration. This study aimed to evaluate the expression profiles of these molecules within heterogeneous circulating tumor cell populations in PC.

A total 40 patients and 10 healthy donors were enrolled. CTCs were isolated from PC patients using Ficoll density gradient centrifugation. Immunofluorescence experiments were conducted with the following combinations of antibodies: (SF25/CD45), (CK/CD45/STIM1) and (CK/CD45/ORAI1). Healthy donors' PBMCs were also evaluated. Biomarker expression was assessed using the automated VyCAP platform, followed by statistical analyses and correlation with clinical data.

Among the 40 patients, only 6 (15%) harbored (CK+/CD45-) tumor cells, whereas all displayed (CK-/CD45-) cells with a size ≥10μm (potentially cancer cells under EMT). Notably, the (CK-/CD45-) subpopulation was elevated in patients' samples compared to healthy donors (AUC,0.8738; p=0.0003), demonstrating strong discrimination ability. Concerning STIM1 and ORAI1 expression, (CK+/STIM1+/CD45-) and (CK+/ORAI1+/CD45-) CTCs were observed in 15% and 2.5% of patients, respectively. Furthermore, among patients harboring (CK-/CD45-) cells, STIM1 was expressed in 74%, ORAI1 in 11% and CD98hc (target of SF25) in 43% of PC patients. Additionally, the presence of (CK+/STIM1+/CD45-) CTCs was correlated with better Overall Survival (OS: log-rank p=0.04, HR=0.164). In contrast, among patients who progressed within one year, the presence of ≥2 (CD98hc+/CD45-) cells was associated with shorter Progression Free Survival (PFS: log-rank p=0.017, HR=8).

This study is the first to characterize the expression of STIM1, ORAI1, and CD98hc in CTCs and (CK-/CD45) cells from PC patients, revealing the heterogeneity of circulating tumor-associated cells and underscoring their potential clinical significance.



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Serglycin regulates C10orf118 function and hyaluronan metabolism to provoke glioblastoma cells' aggressiveness

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Serglycin (SRGN) is the only intracellular proteoglycan, being also found secreted within the extracellular matrix (ECM). It participates in the formation and maturation of secretory vesicles and provokes oncogenic signaling in malignancies, including glioblastoma multiforme (GBM). GBM is the most lethal brain tumor and its formation is accompanied by apparent ECM remodeling, advancing its tumorigenic properties. Hyaluronan (HA) and specifically low-molecular-weight (LMW) HA is a prominent component of GBM microenvironment, initiating diverse signaling cascades to stimulate GBM progression. Recent studies have highlighted the roles of C10orf118 protein in regulating HA synthase 2 (HAS2) levels in breast cancer cells, suggesting a context-dependent interplay between C10orf118 and HA. In this study, we established LN-18^{shSCR} GBM cells, expressing high levels of SRGN, and LN-18^{shSRGN} cells, with depleted SRGN expression. Loss of SRGN led to apparent attenuation of LN-18^{shSRGN} aggressiveness, while notably HAS2 and HA hyaluronidase TMEM2 levels were also diminished, suggesting the deprivation of oncogenic LMW-HA. We also observed downregulated expression of C10orf118 in LN-18^{shSRGN} cells and thus proceeded with transient silencing of its expression through siRNA. Loss of C10orf118 provoked HAS2 transcription and HAS2-antisense 1 (HAS2-AS1) levels, further stabilizing HAS2 mRNA in LN-18^{shSCR}, whereas C10orf118 suppression in LN-18^{shSRGN} cells concluded to reduced levels of the above. Of importance was the finding that SRGN directly interacts with C10orf118 intracellularly. Furthermore, we demonstrated the implication of HAS2 in maintaining the proliferative capacity and potent signaling of GBM cells, through C10orf118 and HAS2 silencing alone. Altogether, our results highlight the versatile role of SRGN in stimulating a dynamic tumor microenvironment, by orchestrating the C10orf118/HAS2 axis and GBM oncogenic properties.

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Discovering New Pathways of Innate Immunity in Breast Cancer Metastasis.

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Neoadjuvant chemotherapy (NACT) represents a well-established therapeutic strategy for breast cancer, particularly for the triple-negative subtype. Despite approximately 30-40% of patients achieving a complete pathological response, a substantial proportion either fail to respond or develop disease recurrence, thereby increasing the risk of metastatic progression. Therefore, the development of new, more effective therapeutic approaches remains a significant challenge. We have previously shown that NACT enhances the secretion of extracellular vesicles from the primary tumour, which target normal cells in distant organs. This intercellular communication engages innate immune components, which in turn promote metastasis formation. Nevertheless, the cellular and molecular mechanisms underlying this process remain largely undefined. Our preliminary data revealed a marked heterogeneity and significant alterations in immune cell populations the lung metastatic niche in response to NACT in breast cancer mouse models. Utilizing state-of-the-art methodologies, including single-cell RNA sequencing and multicolor flow cytometry, we aim to identify, functionally characterize, and selectively deplete or reprogram key myeloid cell subsets within lung metastatic niche in mouse models of breast cancer. Our study focuses on basophils, which have been predominantly studied in the context of allergic responses, while their role in metastatic progression remains essentially unexplored. Our findings suggest that interactions between basophils and other myeloid populations, such as monocytes and macrophages, critically contribute to the modulation of the pre-metastatic microenvironment

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CTCF - Mediated Regulation in Alzheimer's Disease

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Alzheimer's disease (AD) is the leading cause of dementia [1] , and although significant progress has been made in elucidating its pathology, the genomic and epigenomic mechanisms underlying the disease remain insufficiently understood, especially through multi-omic analyses of patient cohorts. Alterations of the CTCF binding and chromatin looping have been observed in AD affecting the regulation of genes linked to synaptic function and neuroinflammation [2]. To explore the relationship between transcriptomic and epigenomic regulation, this study investigates the changes in the binding sites of CCCTC-binding factor (CTCF) across the clinical stages. Using dorsolateral prefrontal cortex samples (Brodmann area 46) from the Rush Alzheimer's Disease cohort [3], CTCF chromatin immunoprecipitation sequencing (ChIP-seq) data were integrated with RNA sequencing (RNA-seq). Differential binding analysis and peak annotation were performed.

Comparisons of CTCF binding across AD stages revealed that the reduction in CTCF occupancy is not global but region-specific. Differential binding of early- and late-stage AD identified over a thousand significantly altered genomic sites, many of which were located in promoters and intronic regions. Integration with RNA-seq uncovered hundreds of differentially expressed genes enriched in synaptic organization and cell adhesion pathways. For specific neuronal genes, it is demonstrated that CTCF redistribution influences transcription in a location-dependent manner, underscoring its role in AD-related regulatory alterations.

In contrast to previous comparisons of controls versus AD patients [4], this multistage approach reveals progressive, site-specific, and adaptive remodeling of CTCF binding. Jointly, the findings suggest that AD progression does not involve a global breakdown of chromatin insulation, but rather dynamic, environment-dependent regulatory adaptations, linking three-dimensional genome remodeling to synaptic and transcriptional dysfunction [5].

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Accumulation kinetics and biological action of doxorubicin in rabbit intervertebral discs

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Doxorubicin (DOX) is widely used for the treatment of several tumors, but considerable dose-dependent side-effects on normal tissues have been reported. However, there is lack of evidence on the bioavailability and biological effect of DOX on the non-vascularized intervertebral discs (IVDs). The aim of the present study was to follow for the first time the kinetics of DOX accumulation/clearance in the IVD, as well as to assess the drug's biological action in the annulus fibrosus (AF) and nucleus pulposus (NP) IVD cells and tissues. DOX was administered intravenously to rabbits and IVDs were isolated at several time-points. A highly sensitive and reliable LC-HRMS/MS analytical method was employed for DOX quantification in IVD tissues in comparison to the plasma and the vascularized skin. *In vitro* experiments were performed to assess the effect of the drug on rabbit IVD cells' viability, intracellular redox status, gene/protein expression and extracellular matrix (ECM) deposition. The effect of DOX administration on IVD tissue quality was studied *in vivo* by histological analysis. DOX distribution was found to differ between the skin and the IVD, but also between the two distinct IVD regions, i.e. the AF and the NP: both IVD tissues retained lower DOX concentrations than the skin, while the NP showed delayed DOX accumulation, as well as elimination, mainly due to the latter's greater distance from blood supply. The low DOX concentrations reaching the IVDs had marginal effects on cells' viability, intracellular redox status and p38 MAPK activation, while they did not evoke cellular senescence. Most importantly, the drug did not negatively affect ECM integrity, as collagen and proteoglycan content remained stable *in vitro* and *in vivo*. Our findings give prominence to the requirement for bioavailability analysis of any traditional or newly discovered drug in additional tissues/organs beyond its specific target, to prevent undesirable collateral side-effects.

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Mass spectrometry–based proteomic profiling of sputum mucins in bronchial asthma: a pilot study

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Mucins MUC5AC and MUC5B are high molecular weight, heavily glycosylated proteins that play a central role in the pathology of bronchial asthma. They contribute to mucociliary clearance and innate epithelial defense, but their pathological overexpression promotes airway obstruction and disease exacerbations. Research into their roles is hindered by challenges in their extraction and analysis due to their size and complexity. This study aims to investigate technical approaches to streamline efficient mucin analysis in sputum samples and explore potential interacting molecules in asthma.

Induced sputum samples from one healthy control and four asthmatic patients were solubilized using four different lysis solutions (Urea/Guanidine, RIPA, Tris/SDS, and RIPA/Tris/SDS). Samples were prepared for mass spectrometry analysis using the FASP (Filter-Aided Sample Preparation) protocol, employing trypsin proteolysis. Additionally, Western blotting was also conducted to detect mucins MUC5AC and MUC5B in the different samples.

While traditional biochemical methods, such as Western blot immunodetection, failed to clearly resolve mucins due to their structural complexity discussed above in the introduction, mass spectrometry-based proteomic analysis successfully identified nine mucins, as well as several other sputum proteins. Key mucins, MUC5AC and MUC5B, were consistently identified and relatively quantified in all samples.

In conclusion, this study presents an optimized and reproducible workflow for the analysis of sputum mucins using mass spectrometry-based proteomics, aiming to simplify and standardize mucin characterization procedures. By enabling the comparative identification of specific mucin levels in different sputum samples and conditions, this approach supports the potential application of mucins as biomarkers in the field of muco-obstructive diseases, including asthma.



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Targeting NR5A2 Reprograms Transcriptional and Metabolic Networks to Suppress Therapy-Resistant Glioblastoma

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Glioblastoma (GBM) is an aggressive primary brain tumor and the most commonly diagnosed malignant neoplasm of the central nervous system. Despite extensive research and current therapeutic interventions, GBM remains essentially incurable and is associated with poor patient survival. These clinical challenges highlight the urgent need for novel therapeutic strategies and molecular targets. We have recently identified the nuclear receptor NR5A2 (also known as LRH1) as a promising therapeutic target in GBM. In the present study, we employed a multidisciplinary approach to evaluate the therapeutic potential of NR5A2 activation and dissect its mechanism of action using patient-derived GBM cells. Our functional analyses demonstrated that NR5A2 activation using well-established agonists (DLPC, RJW100, 6N) reduced the growth of patient-derived GBM cells in vitro and in patient-derived orthotopic xenograft models in vivo. Moreover, using patient-derived GBM cells resistant to temozolomide (the standard-of-care chemotherapeutic agent), we showed that NR5A2 activation remains effective in reducing cell proliferation and growth. Transcriptomic and epigenetic profiling of NR5A2 agonist–treated patient-derived GBM cells revealed that NR5A2 activation significantly downregulated gene programs associated with cell-cycle progression, glycolytic metabolism, epithelial-to-mesenchymal transition, and angiogenesis, while upregulating apoptotic pathways. Metabolic assays further demonstrated reduced glucose and fatty acid utilization, indicating that NR5A2 activation reprograms cancer-associated metabolic pathways toward a less proliferative metabolic state. Furthermore, screening of newly in silico designed NR5A2 agonists identified a new compound that recapitulates the effects of NR5A2 activation on GBM cells. Our findings provide preclinical proof-of-concept supporting NR5A2 activation as a therapeutic strategy for GBM and justify further development of optimized NR5A2 agonists for future clinical translation.

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Nerve growth factor affects energetic pathways in human and mouse pre-adipocytes

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Introduction: Pre-adipocytes are the primary source of white mature adipocytes that store unused energy in the form of triglycerides. Under certain metabolic conditions, white adipose tissue (WAT) expands in size by increasing the number and/or the size of white mature adipocytes. The nerve growth factor (NGF) is known for neuronal development, and its plasma levels are found to be decreased in later stages of metabolic syndrome. Although NGF is expressed in human WAT, little is known about its role in pre-adipocytes.
Aim: The aim of the present study was to investigate the role of NGF in the energetic pathways of pre-adipocytes.
Methods: Human abdominal WAT, donated by patients undergoing general surgery, was used to isolate pre-adipocytes for *in vitro* studies and to measure the release of free-fatty acids in *ex vivo* experiments. Primary human or mouse 3T3L1 pre-adipocytes were incubated with NGF and their mitochondrial activity was measured by MTT and Seahorse assay. Etomoxir was used to block basal fatty acid beta-oxidation and the inhibitor of receptor Trka to block the effect of NGF in pre-adipocytes.
Results: NGF increased the basal release of free-fatty acids in human WAT explants. Human pre-adipocytes incubated with NGF showed significantly increased metabolic activity by MTT. NGF-treated 3T3L1 pre-adipocytes exhibited increased oxygen consumption and glycolysis rates at basal levels but decreased after stress induction in a TrkA receptor-dependent manner. Etomoxir down-regulated basal ATP production in human pre-adipocytes and co-treatment with NGF tended to recover a portion of ATP.
Conclusions: Our data shows the lipolytic properties of NGF in human WAT explants. At the cellular level, NGF increased mitochondrial activity but has a minimal effect on fatty acid beta-oxidation in human pre-adipocytes. In 3T3L1 cells, NGF basally increased the oxygen consumption and glycolysis rates via the TrkA receptor further supporting its functioning role in pre-adipocyte physiology.



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Enforced CYLD Expression Suppresses Proliferation and Promotes Maturation in a CML Cell Line

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Cyld encodes a deubiquitinase (CYLD) that negatively regulates multiple oncogenic pathways, including NF-κB signaling, a key driver of cellular proliferation, survival, and differentiation. *Cyld* expression is frequently downregulated in malignancies, contributing to enhanced proliferative signaling. In chronic myeloid leukemia (CML), leukemic transformation is driven by constitutive tyrosine kinase activity of the BCR::ABL1 fusion protein, which also activates NF-κB. Notably, *Cyld* is minimally expressed in CML patient samples, suggesting that loss of CYLD may contribute to the malignant phenotype. We hypothesized that enforced CYLD expression could attenuate proliferation and influence lineage commitment in CML cells. A doxycycline-inducible *Cyld* expression cassette was introduced into a CML cell line (K562) and pooled populations of selected transduced cells and single-cell-derived clones were established. *Cyld* expression levels were quantified by quantitative-PCR and Western blotting. Proliferation was evaluated and immunophenotype was analyzed by flow cytometry. In polyclonal populations, post selection and doxycycline-induction, CYLD expression increased 5.25-fold over baseline, leading to a modest reduction in proliferation (0.89-fold on day 6). The effect was more pronounced in a single clone (C51) showing markedly elevated CYLD expression (41.9-fold over baseline). Accordingly, western blot analysis confirmed a robust increase in CYLD protein levels in C51. This was accompanied by a 53% reduction in proliferation, without evidence of cell death, suggesting that CYLD may exert antiproliferative effects independent of cytotoxicity. Flow cytometry revealed a differentiation shift, with the $CD36^{-}/CD235a^{+}$ population increasing from 2% to 36%, indicating erythroid maturation and a partial exit from the blast-like undifferentiated state typical of CML cells. These findings demonstrate that enforced *Cyld* expression suppresses proliferation and promotes erythroid differentiation in CML cells that normally lack *Cyld* expression. This supports a tumor-suppressive and lineage-modifying role for *Cyld* in CML and highlights its potential as a target for therapeutic intervention strategies through its reactivation.



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Effects of 3-O-methyl-D-glucose on anxiety-like behavior and activity of acetylcholinesterase isoforms in specific brain regions of pentylenetetrazol (PTZ) epileptic male mice.

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The ketogenic diet, high on fat, moderate on protein, and low on carbohydrates, has positive effects in drug-resistant epilepsy. 3-O-methyl-D-glucose (3MG), a glucose analogue, competes with glucose for transport into the brain but cannot be catabolized to yield energy. This study aimed to evaluate the effects of 3MG: a) on anxiety-like behavior (thigmotaxis time and number of entries in the central area) observed 24 hours after one and two pentylenotetrazole (PTZ)-induced epileptic seizures and b) in the activity of G1 and G4 isoforms of acetylcholinesterase (AChE), determined in the salt soluble (SS) and detergent (DS) fraction, respectively, in brain regions of mice 24 hours after two epileptic seizures. Adult male BALB/c mice were divided into four groups: a) Control group: treatment with 0,9% NaCl, b) PTZ group: PTZ was administered (60 mg/kg, ip) for the induction of epileptic seizures, c) 3MG group: treatment with 3MG (500 mg/kg, ip) and d) PTZ+3MG group: administration of 3MG (500 mg/kg, ip) 30 minutes before PTZ (60 mg/kg, ip) administrations. Behavioral assessments were conducted using the open field test, while AChE activity was determined using Ellman's colorimetric method in both SS and DS fractions. Our results showed that 3MG administration, a single epileptic seizure, or co-administration of 3MG and PTZ had no effect on anxiety-like behavior, whereas after the second seizure, all corresponding groups exhibited anxiety-like behavior. After two epileptic seizures (PTZ group) and 3MG administration (3MG group and PTZ+3MG group) our results showed brain area-specific statistic decreases in the activity of G1 and G4, along with changes in the G1/G4 ratio in most brain regions examined. In conclusion, two PTZ-induced seizures lead to anxiety-like behavior and modulate the cholinergic system. 3-O-Methyl-D-glucose administration appears to also modulate the cholinergic system without inducing anxiolytic-like effects.



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Molecular and phenotypic characterization of a paclitaxel-resistant breast cancer cell line: Insights into chemoresistance in Triple-Negative Breast Cancer

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Breast cancer is a leading cause of death among women globally and is known for its molecular diversity. It is classified into three main subtypes based on the presence or absence of specific molecular markers: hormone receptors (estrogen and progesterone) and human epidermal growth factor receptor 2 (HER2). Each subtype has unique characteristics and responds differently to treatments. Triple-negative breast cancer (TNBC) represents an aggressive and heterogeneous subtype with limited therapeutic options due to the absence of targetable receptors. Consequently, treatment relies primarily on chemotherapy, which frequently encounters drug resistance. Paclitaxel, a commonly used and effective chemotherapeutic agent, often faces resistance in TNBC, leading to poor patient outcomes.

The purpose of this study is to establish and characterize a paclitaxel-resistant cell line derived from the TNBC cell line MDA-MB-231, to investigate the underlying mechanisms of acquired resistance. Characterization of the generated resistant cell line involves evaluating changes in receptor expression profiles and the phenotypic shift between mesenchymal and epithelial characteristics. A comprehensive understanding of these resistance mechanisms is of utmost importance, as it can be an asset on the development of alternative therapeutic strategies to overcome resistance and improve treatment efficacy.



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Advanced Computational Approaches Identify Gene Expression and Extracellular Vesicle Lipid markers in Alzheimer's Disease

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Herein we provide a multi-level molecular perspective on Alzheimer's disease (AD) by integrating analyses of brain-region-specific gene expression and plasma extracellular vesicle lipidome alterations. Transcriptomic profiling across the entorhinal cortex, prefrontal cortex and superior parietal lobe revealed differentially expressed genes linked to tau phosphorylation, RNA metabolism, protein aggregation, neuroimmune activation, and redox imbalance, defining a spatial gene signature underlying selective vulnerability in AD. Analysis of plasma-derived extracellular vesicles among AD patients and control individuals identified alterations in glycerophospholipids, lysophospholipids and sphingolipids across vesicle subpopulations from neurons and astrocytes. Omics-based approaches could highlight connect region-specific molecular changes as well as circulating biomarkers, offering a framework for early detection and spatially informed therapeutic strategies in AD.

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References:
Krokidis et al., Lipidomic analysis of plasma extracellular vesicles derived from Alzheimer's disease patients. Cells, 2024, 13, p.702.



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Neuroprotective Effects of Anthocyanins

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Neurodegenerative disorders are characterized by progressive neuronal loss, neuroinflammation, and oxidative stress. Our recent evidence suggests that dietary anthocyanins in grapes confer neuroprotection through the clearance of existing pathogenic prion protein aggregates and inhibit the formation of new ones. Specific anthocyanins, such as oenin and myrtillin, can upregulate antioxidant defenses and neutralize reactive oxygen species (ROS) generated by prion infections. Additionally, both compounds attenuated lipopolysaccharide (LPS)-induced neuroinflammatory responses by downregulating the expression of pro-inflammatory cytokines interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α). Cell viability assays under LPS challenge demonstrated that both ACNs protected BV2 cells from mitochondrial dysfunction through modulation of the Keap1–Nrf2 signaling pathway and suppression of pro-inflammatory mediators, with oenin exhibiting a more pronounced protective effect. Next, we generated pre-clinical data in diverse murine models, including an LPS-induced endotoxemia model, a neuroinflammation model and a neurodegeneration model. Data suggests that anthocyanins are metabolized by intestinal bacteria into bioactive phenolic compounds with enhanced systemic bioavailability. These microbial metabolites modulate gut microbial composition, promoting beneficial taxa and producing neuroactive molecules that could influence the gut-brain axis. These results highlight their therapeutic potential as neuroprotective agents for mitigating microglia-mediated neuroinflammation and suggest that dietary anthocyanins may represent a valuable strategy for preventing or delaying neurodegenerative disease progression.

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Impact of lipopolysaccharide on hyaluronan turnover in airway smooth muscle cells from COPD patients

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Chronic Obstructive Pulmonary Disease (COPD) is a chronic inflammatory lung disorder characterized by airway remodeling leading to progressive and irreversible airflow limitation. Acute exacerbations of COPD are major events, triggered by bacterial or viral infections that worsen symptoms and contribute to morbidity, mortality, and healthcare burden. Hyaluronan (HA) is a key component of the airways that regulates tissue integrity and inflammation. This study aims to investigate the role of lipopolysaccharide (LPS), a bacterial endotoxin that models bacterial infection-driven exacerbations, on HA turnover by primary airway smooth muscle cells (ASMCs) from COPD patients. Primary ASMCs were established from endobronchial biopsies of COPD patients and non-COPD subjects who underwent diagnostic bronchoscopy and served as controls. Cells were stimulated with LPS (100 ng/mL) for 24 hours. HA secretion was measured by ELISA. The expression of HA synthesizing enzymes (HAS1, 2, 3), HA degrading enzymes (HYAL1, 2, TMEM2), and HA receptors (CD44, RHAMM, TLR4) were assessed by real-time PCR. Overall, LPS stimulation elicits donor-dependent alterations in HA turnover. LPS treatment of ASMCs did not alter HA secretion or gene expression of HAS1, HAS3, HYAL1, and HYAL2, neither in COPD nor in controls. However, in ASMCs from COPD patients, LPS significantly downregulated gene expression of HAS2, TMEM2, CD44, RHAMM, and TLR4. These findings indicate that LPS differentially modulates HA turnover in ASMCs from COPD patients compared to non-COPD controls. The selective downregulation of *HAS2*, *TMEM2*, *CD44t*, *RHAMM*, and *TLR4* in COPD-derived ASMCs suggests an impaired HA synthetic and signaling response to bacterial stimuli. This dysregulation may contribute to altered airway remodeling and defective resolution of inflammation during infection-driven COPD exacerbations. Collectively, the data support the concept that aberrant HA metabolism in ASMCs represents a potential mechanism linking bacterial exposure to persistent airway inflammation and remodeling in COPD.



P215 - 295

Synthesis and biological evaluation of novel imatinib derivatives as potential tyrosine kinase inhibitors.

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May 2001 marked a hallmark in drug discovery as Imatinib-the first tyrosine kinase inhibitor-was approved by the FDA. Imatinib selectively targets the chimeric BCR-ABL protein which is highly expressed in Chronic Myelogenous Leukemia (CML) as well as PDGFR, c-KIT, and CSF in other types of cancer such as GIST. In this study newly synthesized Imatinib analogs were evaluated as potential TKIs. The synthetic process of the drug involves the removal of piperazinyl ring (ring E) of Imatinib followed by modification to the terminal phenyl ring by adding the bromo, nitrogen dioxide and trifluoromethlyl group in different positions on the D ring. These analogs were tested in two different breast cancer cell lines MCF-7 and MDA-MB-468 in a dose dependent manner for 24,48 and 72 hours respectively using the MTT assay to calculate IC-50. In addition, U2OS osteosarcoma cells were treated with two other classes of Imatinib analogs, featuring a urea moiety addition and the inversion of the amide bond of the parent compound while maintaining some changes in the D-ring to evaluate potential inhibition due to PDGFR signaling. Then analogs showing the lowest IC-50 values were further investigated to explore their role in the cell cycle arrest and apoptosis by flow cytometry. Ultimately, Immunoblotting was performed to evaluate the alterations on the protein level and which growth factors and signaling molecules are affected by this tyrosine kinase inhibitors.



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Functional analysis of Abracl protein in Neuro2a cell line

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Abracl is a small, highly conserved protein expressed in all eukaryotic organisms, except fungi. Previous studies from our laboratory using the neuroblastoma cell line Neuro2a, including both Abracl-overexpressing and Abracl-knockout clones, have demonstrated that Abracl is associated with cellular proliferation and migration. Notably, knockout cells exhibited a significant increase in size. These findings led us to further investigate the role of Abracl in cell cycle regulation and cytoskeletal organization. To elucidate these roles, we performed both flow cytometry and immunoassays to assess differences in cell cycle distribution between overexpression and knockout clones, and immunoassays to evaluate changes in cytoskeletal protein expression. Our results revealed that Abracl-overexpressing and Abracl-knockout cells accumulate in different phases of the cell cycle. Additionally, differences were observed in the expression of cytoskeletal components. In conclusion, our findings indicate that in Neuro2a cells, Abracl regulates cell proliferation through modulation of the cell cycle and may additionally contribute to the organization of the cytoskeleton.

Key-words: Abracl, Neuro2a, Cell Cycle, Cytoskeleton



P217 - 303

siREN: A Dual RNAi Therapeutic Strategy with Targeted Lipid Nanoparticle Delivery for Chronic Lymphocytic Leukemia

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siREN, developed for the iGEM Thessaloniki 2025 competition, is a dual RNA interference (RNAi) therapeutic strategy for Chronic Lymphocytic Leukemia (CLL). It employs a targeted lipid nanoparticle (LNP) to deliver siRNAs against *BTK* and *BCL2* genes, key regulators of CLL cell survival and proliferation, to induce cell death in malignant B cells.

The project combines *in silico*-modeled LNPs encapsulating experimentally validated and newly designed siRNAs with wet lab validation. Computationally, we developed machine learning models for siRNA efficacy prediction and performed thermodynamic and structural analysis of the siRNA-target mRNA-Ago2 complex, selecting the most promising siRNAs for experimental validation. LNP formulation was modeled, and the optimal ROR1-targeting antibody for cell-specific delivery was identified and evaluated using Rosetta InterfaceAnalyzer. Lipid components were visualized using OpenBabel and Avogadro2 and bilayer structures were simulated with the CHARMM-GUI Membrane Builder Tool. Experimentally, for proof of concept, we transfected MEC-1 CLL cells with a literature-validated BCL2 siRNA, testing BCL2 mRNA/protein levels.

Fluorometer readings verified efficient intracellular uptake. qPCR and immunoblot analyses demonstrated a trend of dose-dependent reduction in BCL2 mRNA/protein levels, supporting the functional efficacy of the RNAi component. Our preliminary wet lab results support the potential of the siRNA payload for integration into the computationally designed, ROR1-targeted LNP platform. ROR1 ligand, a receptor abundantly expressed on CLL cells, is largely absent in healthy tissues, and ROR1-targeted LNP platform aims to maximize specificity and minimize off-target effects.

siREN project successfully established a proof of concept by combining robust *in silico* design with experimental validation. This integrated approach provides a strong foundation for developing a precise and effective dual-RNAi therapy for CLL.



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Proteins involved in endocellular trafficking in eukaryotic cells: comparative genomic analysis to identify orthologs in prokaryotes

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The endomembrane trafficking system is a characteristic feature of eukaryotic cells, through which endocytosis and exocytosis of macromolecules, such as proteins, take place, as well as the transport of molecules among membrane-bound organelles for their recycling to and from the plasma membrane. The organelles involved in this system are the endoplasmic reticulum, Golgi apparatus, lysosomes, endosomes and the plasma membrane. Specifically, trafficking occurs via membrane vesicles, in which molecules are packaged and transported within the cell with the assistance of the cytoskeleton. This process involves numerous proteins, each with a distinct functional role. A fundamental difference between prokaryotic and eukaryotic organisms is the absence of membrane-bound organelles in prokaryotes. As a result, bacteria and archaea lack an endomembrane trafficking system. Nevertheless, despite the absence of this system in prokaryotes, individual orthologous proteins involved in membrane trafficking have been identified in various prokaryotic lineages. However, no single prokaryotic organism has been reported to possess all of these orthologous proteins. The identification of an organism that harbors a substantial number of these proteins could indicate a putative ancestor of the endomembrane trafficking system.

The aim of this study was to identify orthologous proteins that show homology to eukaryotic protein factors involved in the endomembrane trafficking system, across major prokaryotic phyla, classes and selected genera of interest. Specifically, bioinformatic analyses were conducted for twenty-three prokaryotic proteins previously reported to have eukaryotic homologs, using the NCBI Clusters of Orthologous Genes (COGs) database and the KEGG Orthology (KO) database. In addition, BLAST analyses were performed to identify protein homologs. The analysis revealed prokaryotic organisms, such as members of the bacterial class Actinomycetes, that harbor a large number of the examined proteins. These results suggest that the identified organisms could represent putative prokaryotic ancestors of the eukaryotic endomembrane trafficking system.



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Comparative proteomic analysis of bone marrow CD138+ cells to understand the progression of plasma cell dyscrasias

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Multiple myeloma (MM) is a plasma cell dyscrasia that remains incurable despite major therapeutic advances, showing a heterogeneous clinical course with frequent relapses and complications. It develops through asymptomatic precursor stages, Monoclonal Gammopathy of Undetermined Significance (MGUS) and smoldering Multiple Myeloma (sMM), which may persist for years before progressing to symptomatic disease. Despite improved understanding of MM biology, the molecular mechanisms underlying disease onset and progression remain incompletely defined, underscoring the need to identify early drivers and potential therapeutic targets.

Proteomic analysis has emerged as a powerful tool for investigating disease-associated signaling alterations and uncovering molecular determinants of progression. Building on this approach, we performed a comparative proteomic analysis of 94 $CD138^+$ plasma cell samples across disease stages using LC-MS/MS. An optimized sample preparation method enabled use of the leftover organic fraction from nucleic acid isolation, minimizing sample requirements and leading to the identification of 6,204 proteins.

Pairwise comparisons revealed significant differences between MM vs. MGUS and MM vs. sMM, while no significant changes were observed between sMM vs. MGUS, reflecting their relative clinical similarity. Among the significant proteins, 370 showed monotonous changes across the three disease stages, being consistently up- or downregulated. Pathway analysis indicated that upregulated proteins were mainly involved in mRNA processing, ribosome biogenesis, and DNA replication, supporting enhanced gene expression and cell proliferation, while downregulated proteins were enriched in immune response functions, indicating progressive immune attenuation. Proteins with monotonous changes were further examined through co-expression network analysis to assess coordinated expression correlation changes across stages. The analysis identified proteins showing both differential expression and dynamic network behavior, including known (e.g., EZH2, SUMO2, MMP9, PTBP1) and novel factors associated with MM.

Collectively, these findings offer insight into key molecular mechanisms of MM progression and lay the groundwork for further investigations in systems biology.

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Large-scale collaborative work using statistical and AI tools to collect and organize stem cell differentiation protocols

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The increasing complexity and lack of standardization in cell differentiation protocols often limit reproducibility and comparative interpretation in stem cell research. These protocols, usually reported in narrative and heterogeneous formats, make it challenging to identify methodological patterns or key differentiation steps. This study presents an artificial intelligence (AI) driven system designed to organize, summarize and interpret differentiation protocols of embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs). The system utilizes a Chroma based vector database to store protocol data as document chunks and corresponding embeddings. Each protocol is treated as a separate collection, enabling targeted retrieval and analysis through a Retrieval Augmented Generation (RAG) framework. The pipeline includes sequential modules for summarization, classification, and reconstruction of the differentiation process. Summaries are generated from paired text segments using a large language model (LLM), focusing on experimental objectives, reagents, and procedural flow. Each summary is then classified into one of three categories based on contextual cues: undifferentiated cells, differentiation process or differentiated cells. The outputs are subsequently sorted, merged, and refined to form a structured differentiation protocol that includes cell line identification, stage-specific descriptions and estimated durations. Performance evaluation demonstrated high accuracy and consistency across all stages of the pipeline, confirming its reliability for large-scale data organization. Overall, this work demonstrates the utility of AI for transforming unstructured biological documentation into organized, machine-readable data. The proposed system provides a reproducible and scalable framework for managing complex differentiation data and can be extended to other experimental workflows within molecular and cellular biology.



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Cell-Corr: a user-friendly graphical software for descriptive analysis of molecular dynamics with live-cell imaging

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Understanding mechanisms underlying complex intracellular processes often requires studying the dynamics of specific molecules or structures. For instance, researchers can assess the influence a protein has on a dynamic structure just by comparing the dynamicity of the structure between wild type and a mutant organism for the specific protein. These measurements can be typically deduced from live-cell time series imaging of the structure of interest extrapolating quantitative terms of similarity between consecutive frames. One of the most commonly used factors for image comparisons is Pearson's Correlation Coefficient (PCC). This statistical tool can provide a descriptive measure for studying the dynamics of inner cell components such as the actin cytoskeleton and the endoplasmic reticulum. Although this technique is very helpful, there is not available software for a complete analysis and in all cases, researchers rely on multiple programs like Fiji and Excel for such an experiment or use scripts in MATLAB that require programming expertise. Here, we present Cell-Corr, a new user-friendly software based on python, for image correlation analysis using PCC, designed to be more effective, less time-consuming and with built-in statistical testing and plotting. The capabilities of Cell-Corr are demonstrated via two examples: (1) the analysis of the effects of SMIFH2 and CK666 two inhibitors for the actin nucleator families, formins and ARP2/3, on *Arabidopsis* plants marked with LifeAct-GFP and (2) by testing how Latrunculin B changes the dynamics of endoplasmic reticulum on plants stably expressing HDEL-GFP.



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CpGene: a web application for epigenetic biomarker identification from DNA methylation arrays

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Background: DNA methylation (DNAme) remains the best studied epigenetic mechanism that describes bilateral changes which affect gene expression without altering the DNA sequence and has been correlated with tissue differentiation/specificity. While highthroughput techniques have been developed to describe candidate biomarkers of epigenetic disruption that account for numerous diseases (e.g. cancer, metabolic disorders), in-depth bioinformatic analysis often requires specialized expertise and a multidisciplinary computational team. To address this limitation, we developed *CpGene*, a user-friendly web application designed for comprehensive analysis of DNAme array data (e.g., Illumina 450K, EPIC v1.0). *CpGene* enables provides an accessible and integrative analytical platform that enables both basic scientists and clinicians to conduct comprehensive DNAme analysis without the need for extensive bioinformatics expertise.

Methods: The core architecture of CpGene was developed using well-established and widely adopted software packages to ensure robustness, transparency, and reproducibility. The *minfi* R package was employed for data preprocessing, quality control, and generation of beta value matrices. Differential methylation analysis was implemented using both traditional statistical approaches from the *limma* package and machine learning algorithms available through the *scikit-learn* Python library. For downstream interpretation, CpGene integrates functional enrichment analysis using the *Enrichr* API, enabling users to directly explore biological pathways and gene sets associated with methylation changes.

Results/Conclusions: *CpGene* offers an intuitive and interactive environment for preprocessing, normalization, quality control, feature ranking, differential analysis, gene set enrichment, and data visualization. Its dynamic interface supports multiple analysis strategies and produces publication-ready figures, including bar, scatter, and volcano plots. By consolidating complex analytical workflows into a single accessible platform, CpGene minimizes technical barriers, promotes reproducibility, and facilitates the discovery of biologically and clinically relevant CpG sites and genes. This integrative tool represents a valuable resource for researchers seeking to uncover disease-associated epigenetic alterations and identify actionable methylation-based biomarkers without the need for extensive bioinformatics expertise.



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Rapid remodeling of the human gut microbiome in response to short-term animal product restriction

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Diet strongly influences the gut microbiome, which in turn influences health, yet the effects of dietary patterns on microbiome composition and function remain underexplored in humans. We profiled a unique group of apparently healthy individuals from Greece, who alternate between omnivory and restriction of animal products for religious reasons (periodically restricted group, N=200), as well as a continuously omnivorous group in parallel (non-restricted group, N=211). Using 16S rRNA sequencing, plasma metabolomics, and proteomics, we assessed the impact of three-to-four weeks of dietary restriction on gut microbiome composition and function and also explored links with host plasma biology. Animal product restriction was found to reduce microbial diversity, primarily affecting rare taxa, and altered the abundance of nearly one-third of bacterial genera. Functional shifts included downregulation of cholesterol biosynthesis and purine degradation pathways, alongside upregulation of microbial biosynthesis of vitamin B2 and tryptophan, suggesting compensatory microbial responses to dietary nutrient depletion. Multi-omics integration revealed four microbial-metabolite-protein clusters, including a diet-responsive module linking *Negativibacillus* with potent metabolic regulator FGF21 and intermediate-density lipoproteins. Our findings demonstrate rapid adaptive plasticity of the human gut microbiome in response to short-term dietary restriction and highlight candidate microbial and molecular pathways that may mediate effects of animal product restriction on health.



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Short-term animal product dietary restriction alters metabolic profiles and modulates immune function

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Dietary interventions are powerful tools for disease prevention and health promotion, yet the molecular mechanisms by which diet influences health remain incompletely understood. Investigating the effects of diet in healthy individuals enables characterization of molecular and physiological responses in the absence of disease-related confounders and facilitates the identification of diet-responsive pathways underlying physiological regulation. We investigated the immunometabolic effects of short-term dietary restriction of animal products in a unique group of apparently healthy individuals (N=200) who alternate between omnivory and animal product restriction for religious reasons. We profiled clinical biomarkers and immune parameters during both dietary states, alongside a control group of continuously omnivorous individuals (N=211). Short-term restriction was associated with reductions in total and non-high-density lipoprotein cholesterol, urea, creatinine, alanine aminotransferase, and gamma-glutamyltransferase, and a concurrent 73% reduction of normal-range C-reactive protein levels. Immune profiling revealed reductions in frequencies of non-classical monocytes, $CD56^+$ natural killer cells, and $CD8^+$ memory T cells, accompanied by an increased response of anti-inflammatory cytokine IL-10, suggesting a shift toward a less inflammatory immune state. Although most changes were in a direction suggesting beneficial health effects, levels of alkaline phosphatase increased upon restriction, implying possible negative effects on bone turnover or liver function. Short-term animal product restriction mostly improves immunometabolic health markers and may lower chronic inflammatory disease risk. Our findings highlight the value of studying diet in the absence of disease to reveal adaptive molecular changes and emphasize the translational potential of short-term dietary interventions in altering health-related risks.



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Kinetic Modelling of Light Mediation for Glucose-Stimulated Insulin Release in Human Pancreatic Beta-Cells

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The islets of Langerhans regulate blood sugar by secreting hormones from alpha- and beta-cells, including glucagon and insulin. [1]. Their balanced release is key to blood glucose regulation [2], with dysregulation linked to metabolic syndrome and diabetes. Improving glucose-stimulated insulin secretion (GSIS) in exogenous pancreatic β-cells is a promising biotechnological avenue to lessen the number of beta-cells needed to treat diabetes [3]. Optogenetics, which afford high spatiotemporal control of cellular functions and are not limited by pharmacological agent diffusion or clearance rates, have been successfully employed to boost GSIS [3]. Upon delivery of a human codon-optimized blue light photoactivatable adenylyl cyclase (bPAC) to human beta-cells, illumination with blue light induces a statistically significant 10-fold increase of cyclic adenosine monophosphate (cAMP) versus the base case (no illumination), with a concomitant 3-fold rise in GSIS. Both on- and off-kinetics (as $[cAMP]_i$ and secreted insulin concentrations) were measured with and without illumination, respectively.

The challenge, however, is to acquire a mechanistic, quantitative understanding of how illumination triggers bPAC to promote cAMP production and GSIS, based on observable intracellular dynamic variables for which time-series data are available [3]. A recent mathematical model [4] predicts both the transient and steady-state profiles of secreted insulin and glucagon, including the biphasic response of normal beta-cells to hyperglycemia, but does not consider the effects of blue light on cAMP or the extent of GSIS.

The present paper presents the structure and parameter estimation implementation [5] of a simple, linear biochemical reaction network (and ODE system) developed to capture this photobiochemical sequence, with specific emphasis on the (hitherto unknown) time lag between the on- (illumination) and off- (dark) phase of cAMP-induced GSIS. The ultimate goal is an articulated model to guide Design of Experiments (DoE) under a variety of light (duration, intensity) and chemical (e.g., glucose) conditions.

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P226 - 93

Microbial bioenergetic diversity analysis based on 16SrRNA (amplicon) metagenomic data from soil samples

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Metagenomics is a constantly evolving scientific field that has enabled the study of microbial diversity direct from environmental samples, without the need for cultivation. Sequencing data from the 16S rRNA gene region are widely used to study bacterial diversity. Soil represents one of the most complex and diverse microbial habitats. Bacteria are abundant in soil participating in numerous metabolic processes promoting essential ecosystem functions including nutrient cycling, organic matter decomposition and plant growth. Despite their ecological importance, the differences in soil microbial communities across ecosystems remain poorly understood. In this study, we focused on publicly available 16S rRNA (amplicon) sequencing data from soil samples collected across different vegetation zones of Crete island[1], characterized by their elevation above sea level. We explored patterns of bacterial diversity according to vegetation zone, focusing on a set of species representing the variety of prokaryotic bioenergetic pathways[2]. Our results showed that heterotrophic bacteria dominate the soil communities, with actinobacteria and proteobacteria being the most abundant phyla. Certain bacterial genera appeared in higher frequency in specific vegetation zones (e.g. *Marinobacter*, *Acidiphilium*). We found that the lowland zone (20-300m) displays significantly higher species richness compared to other zones when we calculated the alpha diversity indices Observed OTUs and Chao1 ($p \leq 0,05$). However, no significant differences were observed in Shannon and Simpson indices, suggesting that species diversity and evenness remain relatively stable across vegetation zones. Beta diversity analysis indicates that bacterial community composition was similar across vegetation zones, except in coastal and littoral zone, likely reflecting unique environmental conditions (salinity). This study demonstrates the consistency of the soil bacterial diversity across vegetation zones, while indicating that local environmental conditions can shape species' richness and allow certain bacterial genera to be more prevalent in specific ecozones.

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P227 - 97

In silico antimicrobial peptide discovery in the Animal kingdom

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Antibiotic resistance, a pressing public health issue, urges for the discovery of novel antimicrobial agents. Antimicrobial peptides (AMPs), ubiquitous small amino acid sequences, cleaved from larger precursor proteins, are part of the innate immune response of all organisms and a promising source of novel potential therapeutics. The increasing availability of genomes and transcriptomes coming from the animal kingdom, provides an untapped resource of sequence space for the discovery of putative novel AMPs.

In this work we present a custom pipeline, that screens eukaryotic genomes and transcriptomes from the MATEdb2 database, filters for small proteins which are then *in silico* cleaved, isolating the "mature" peptides. Each resulting peptide is classified by multiple state-of-the-art models as AMP or non-AMP. The final list of candidates is characterised by multiple confidence score levels, determined by the consensus among the different classifiers.

To further investigate the resulting high-confidence candidates, 3D structures were predicted. Sequences and structures were compared with those of natural AMPs in all the available databases to identify novel ones, as well as to catalogue domains contained in their larger structure.

Our pipeline shows promising results, predicting 10.480 AMPs, 6.685 of which are novel. After grouping the candidates into families, our work essentially doubles the AMP family space, with the potential for more when scaled to thousands of genomes and transcriptomes.



P228 - 107

Ancient population dynamics and regional patterns of SERPINA1 pathogenic variants related to α1-antitrypsin deficiency (AATD)

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SERPINA1 gene encodes α1-antitrypsin (AAT), a protease inhibitor, essential for protecting lung tissue from neutrophil elastase–mediated damage. Deficiency of AAT (α1-antitrypsin deficiency, AATD) leads to increased risk for early-onset pulmonary emphysema and liver disease, representing a rare but clinically significant genetic disorder. Interestingly, several rare AATD-associated pathogenic variants occur at relatively higher frequencies in modern Greek patients, raising questions about their evolutionary history and the potential influence of past demographic phenomena on their distribution. To address this, we examined 10 known pathogenic SERPINA1 variants reported at high frequencies in Greek AATD patients in a dataset of 1,050 ancient European genomes, spanning the Paleolithic to Early Modern periods. Ancient genomes were initially screened for sequencing reads overlapping the targeted variant positions. Sixty-two genomes showed such overlaps and were subjected to SNP calling to assess genotype quality and verify potential variant presence. Among these, seven genomes exhibited tentative evidence of pathogenic alleles, however, none satisfied the required reliability thresholds for confident identification, reflecting the inherent limitations of ancient DNA data. To overcome these constraints and further investigate the potential demographic factors underlying the modern distribution of these variants, we next employed a simulation-based demographic model to explore how historical population dynamics could have shaped present-day allele frequencies. Using a simplified two-population simulation model, we explored how past demographic events may have influenced the distribution of SERPINA1 variants across Northern and Southern European populations. The simulations reproduced regional patterns, with more rare variants in the South and distinct high-frequency mutations in the North. These findings suggest that historical demographic processes likely played a major role in shaping present-day SERPINA1 variation. These results should be interpreted cautiously, as the model is simplified and does not account for gene flow or migration.



P229 - 158

Epitranscriptome editing and transcriptome fragmentation set immune-related epigenetic memory of monocytes in human systemic lupus erythematosus (SLE)

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Monocytes play versatile roles in inflammation regulation, immune activation and are key effector cells in human systemic lupus erythematosus (SLE). Overproduction of cytokines, self-antigen presentation and amplification of tissue inflammation represent outcomes of transcriptional rewiring within the inflammatory milieu of the disease.

This study aimed to delineate the altered transcriptome of monocytes in SLE, investigate how epitranscriptomic RNA editing affects their function, and assess whether transcriptional perturbations are linked to changes in chromatin organization.

Monocytes from 8 SLE patients (SLEDAI >8) and 5 healthy controls were isolated from PBMCs by Ficoll centrifugation and CD14+ selection. Single-end 75-bp mRNA sequencing was performed, and differential expression was analyzed using edgeR (p-value ≤ 0.05, fold change >1.5), followed by pathway enrichment analysis. A trained immunity signature was constructed from public data and compared with our dataset. A-to-I RNA editing events were profiled with JACUSA2, retaining sites (score > 2.5) with significant differences in editing frequency. Domains of Coordinated Expression (DCEs), defined as genomic regions of consecutive chromosomal bins with gene expression correlation above the healthy average, were estimated to examine whether altered gene expression patterns correspond to chromatin organization changes.

A total of 1,368 differentially expressed genes (DEGs) were identified. Upregulated DEGs were enriched in pathways related to biotic stimulus response, cell chemotaxis and T-cell differentiation. Our genes showed a correlation with trained immunity. Interestingly, ADARB1, a key regulator of RNA editing, was upregulated, and SLE monocytes exhibited significantly increased A-to-I editing frequencies, particularly in immune-related genes such as ARPC2, IL17RA, TLR2, and OSCAR. DCE analysis revealed extensive redistribution of chromatin co-expression activity, indicating rearrangement of transcriptional coordination.

These findings highlight transcriptional changes in SLE monocytes, with RNA editing as an additional layer of immune-related epigenetic memory, while chromatin disorganization amplifies transcriptional dysregulation, further reinforcing the autoimmune phenotype.



P230 - 170

Evolution of apoptosis in early eukaryotes

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Apoptosis is an irreversible process of programmed cell death during which the cell and chromatin shrink while the mitochondrial membrane is disrupted, resulting in the destruction of the cell and the formation of apoptotic bodies. Apoptosis has been studied extensively in animals, but it's evolution in other eukaryotic lineages, including unicellular eukaryotes is largely unknown. In this study, we searched for orthologs of 79 proteins, involved in apoptosis, in the complete genomes of 36 eukaryotic organisms. The ortholog search was performed using information from the SIGNOR, Genecards, PANTHER and Uniprot databases. Additional homology searches were done using BLASTp. Results were evaluated based on E-value, % identity and query cover. For ambiguous results, reverse BLASTp was used to confirm the best reciprocal hit between the orthologs. The proteins selected represent three major groups: 51 proapoptotic proteins, divided into 27 families; 16 antiapoptotic proteins divided into 8 families; and 12 BCL-2 family members (9 proapoptotic and 3 antiapoptotic). The results show that most proteins are conserved in the metazoa, but a notable number of proteins are conserved throughout all lineages, for example members of the IAP, ASPP, and PDCD5 families. The results reveal a minimal apoptosis pathway conserved in early eukaryotes. In this context, relevant implications for the role of apoptosis in unicellular organisms and possible therapeutic projections are discussed.



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Three-Protein Signature for Advanced Liver Fibrosis: A Minimalistic Shallow-Deep Neural Network Approach in MASLD Using Open Proteomics

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Background: Steatotic liver disease affects 20-30% of the global population and continues to rise among individuals with metabolic dysfunction–associated steatotic liver disease (MASLD). Non-invasive detection of advanced liver fibrosis (ALF; F3-F4) remains clinically challenging, as liver biopsy is invasive and imaging-based fibrosis assessment offers limited sensitivity at early stages. Leveraging open-science proteomics and precision machine learning, we investigated whether a minimal biomarker set combined with a lightweight neural network could reliably detect ALF in MASLD patients [1-2].

Methods: We analyzed open biopsy-validated data from 191 MASLD patients profiled by SomaScan v4.0 (4,730 proteins). A non-linear feature-selection framework integrating inductive learning and feature engineering identified three fibrosis-related proteins. A shallow-deep neural network (s-DNN; 3 layers: 23-23-7 neurons) was trained using 10-fold cross-validation (10CV), with an independent test set (~90/10 split) for external evaluation. Performance metrics included ROC-AUC, sensitivity, specificity, and F1-score. Explainable AI methods were applied to confirm biological plausibility of selected biomarkers.

Results: Three proteins: ADAMTSL2, MFAP4, and GDF2 [3-5] emerged as the most informative biomarkers for ALF. ADAMTSL2 participates in extracellular matrix remodeling, MFAP4 contributes to fibrogenesis, and GDF2 regulates hepatic metabolic signaling and angiogenesis. The model achieved strong and consistent performance:

10CV: ROC-AUC 0.82±0.13; Sens 0.88±0.14; Spec 0.76±0.21; F1 0.79±0.13
Test: ROC-AUC 0.83; Sens 0.75; Spec 0.91; F1 0.80

Conclusion:

A minimalistic three-protein panel combined with a lightweight s-DNN enabled high diagnostic accuracy for ALF in MASLD, even in a limited-sample setting. These results highlight the potential of open proteomics and compact AI architectures for creating scalable, minimally invasive precision tools in hepatology and support further validation in larger, independent cohorts.



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Shotgun metagenomics and volatilomics analysis reveal producer-specific microbial and functional patterns in PDO Sfela cheese

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Microbial composition plays a crucial role in shaping the safety, quality and overall identity of artisanal cheeses, and numerous bioinformatics strategies have emerged for effective handling research data. In this study, a multi-omics approach was applied to investigate the Protected Designation of Origin (PDO) Sfela cheese from three independent producers, combining shotgun metagenomics and Gas Chromatography-Ion Mobility Spectrometry (GC-IMS) volatilomics to link the microbiome with functional and sensory attributes. Taxonomic profiling using Kraken2 revealed that the first producer was dominated by *Streptococcus thermophilus* and exhibited low microbial diversity, while the third producer was characterized by *Lactiplantibacillus plantarum*, alongside various non-starter lactic acid bacteria (NSLAB). The second producer showed an intermediate microbial composition. Assembly and binning enabled the construction of high-quality bins representing both dominant and less abundant taxa. KEGG annotation indicated a shared pattern of major metabolic pathways, while broader profiles of CAZY and MEROPS groups were detected in the samples with higher microbial diversity. Volatile fingerprints obtained from GC-IMS, with additional statistical analysis, discriminated the producers and identified certain compounds as major biomarkers contributing to differentiation. Correlation networks (Spearman, bootstrapping, LOO, FDR-filtered) were constructed to explore the microbial associations, the volatile-volatile relations, and taxa-volatile interactions. The microbial network highlighted positive correlations among most NSLAB, and multiple negative correlations characterizing *S. thermophilus*, consistent with its competitive dominance. The volatile network revealed specific relations pointing towards the biochemical pathways co-existing within the cheese matrix. Finaly, taxa-VOCs interactions represented a complex network, supporting the need to decode the mechanisms underpinning the impact of microbiota on cheese sensory attributes. Beyond providing new insights into Sfela's unique characteristics, this study underscores the impact of producer-specific practices on microbial ecology and the consequent cheese profile. It also suggests a robust bioinformatic pipeline for biodiversity, functional and flavor assessment in complex ecosystems.



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Drug-specific signatures of chemoresistance: A metanalysis

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Chemoresistance is the innate or acquired ability of cancer cells to evade the cytotoxic effects of chemotherapeutic agents. It is a major barrier to successful cancer treatment, leading to poor outcomes and disease progression. Understanding the molecular mechanisms of chemoresistance is crucial to effectively develop strategies to predict, prevent and overcome it. This study presents a comprehensive meta-analysis of transcriptomic datasets associated with acquired chemoresistance to major drug such as doxorubicin, cisplatin and gemcitabine. Our aim was to identify common and drug-specific gene expression patterns between resistant cancer cells and their sensitive progenitors. Publicly available microarray datasets from the Gene Expression Omnibus (GEO) database regarding breast, ovarian, neuroblastoma, bladder and gallbladder chemoresistant cell lines were chosen for comparison. Differential expression analysis and functional enrichment analysis using R and Bioconductor packages revealed distinct and overlapping signatures of chemoresistance across the different cancer types and drugs. Key findings include the identification of both universal and drug-specific differentially expressed genes (DEGs) linked to chemoresistance. For doxorubicin resistance in breast cancer, ABC transporters were upregulated, highlighting pathways linked to adhesion, drug efflux, and survival. Gemcitabine resistance signatures in bladder and gallbladder cell lines further proved the involvement of growth factors, adhesion, as well as stress-related pathways. Additionally, cisplatin resistance in ovarian cancer cells pointed out that the DEGs *LAMA2* and *SOX8* were involved in the resistance across all different datasets. In neuroblastoma and ovarian cancer cell datasets, shared DEGs such as *ABCB1, RUNDC3B, CROT, TP53TG1* were involved in the resistance phenotype, while *TMEM47* was the only DEG present in all breast cancer, neuroblastoma and ovarian cancer datasets. These shared and unique gene expression patterns provide potential biomarkers and therapeutic targets to overcome chemoresistance. This study highlights the need for meta-analysis of even larger datasets for understanding and treating drug resistance in cancer.

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Bioinformatics Study on Idiopathic Central Precocious Puberty

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Bioinformatics Study on Idiopathic Central Precocious Puberty

Precocious puberty is a phenomenon of increasing scientific and social interest, as it significantly impacts children's development and health. This study examines the range of factors associated with Central and Peripheral Precocious Puberty, focusing on Idiopathic Central Precocious Puberty and the GnRH (Gonadotropin-Releasing Hormone) axis, which serves as the central regulator of pubertal onset. Through the combined analysis of genetic data, endocrine markers, and clinical observations, the work explores the complex interactions that lead to the premature activation of reproductive mechanisms. Particular attention is given to the application of a variety of bioinformatic tools, which enable comprehensive data evaluation and contribute to the identification of novel genes and molecular pathways related to precocious puberty. The presentation of results aims to highlight the complexity of the condition while emphasizing the importance of a multidisciplinary approach for accurate diagnosis and effective patient management. The proposed framework paves the way for future research and reinforces the application of bioinformatic analyses in pediatric endocrinology, linking theoretical knowledge and clinical practice in a cohesive and practically valuable manner.



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AI-Guided Design of *de novo* Mini-Protein Binders Targeting Allosteric Sites of MALT1 for Cancer Immunomodulation

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MALT1 (Mucosa-Associated Lymphoid Tissue Lymphoma Translocation Protein 1) is a key mediator of NF-κB signaling, and dysregulation of its activity is implicated in various hematological malignancies and solid tumors. Given its central role in both tumor cells and immune regulation, MALT1 inhibition has emerged as a promising therapeutic strategy (1), especially the development of selective, reversible allosteric inhibitors. While small-molecule inhibitors have been explored (2), the design of high-affinity protein-based inhibitors remains a challenge. We present a computational pipeline integrating state-of-the-art AI-driven protein design methods to generate and validate novel mini-protein binders targeting two distinct MALT1 interfaces. Our approach leverages RFdiffusion (3) to generate *de novo* backbone scaffolds designed to bind to MALT1, ProteinMPNN to assign sequences to these binders, followed by structural validation with AlphaFold3 to assess whether the designed binders adopt the intended conformation when modeled in complex with MALT1. Further filtering based on key structural confidence metrics ensures selection of high-confidence binders. We applied this pipeline to two allosteric sites on the apo state of human MALT1: the paracaspase-Ig3 interface (site 1) and one within the paracaspase domain (site 2), generating 1924 and 2660 binders respectively. The designs targeting the two sites were assessed using predefined filtering criteria, indicating their potential as viable MALT1 allosteric modulators, with site 2 yielding a higher success rate. Specifically, only 1.5% and 2.9% of designs, respectively, met stringent criteria for both binder folding and placement, with 13 and 34 designs (0.68% and 1.28% respectively) passing all filtering thresholds. This study underscores the feasibility of AI-driven protein binder design, providing a framework for developing novel protein therapeutics against challenging drug targets like MALT1. Future work will focus on scaling up the number of designs, implementing further filtering criteria related to protein expression and stability, and *in vitro* validation of the top-ranked binders.

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Longitudinal analysis of gut microbiome dynamics following allogeneic hematopoietic stem-cell transplantation: preliminary results using Oxford Nanopore full-length 16S rRNA sequencing

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Hematopoietic Stem Cell Transplantation (HSCT) is a standard, life-saving treatment for patients with hematologic malignancies. Allogeneic HSCT is associated with significant alterations in gut microbiota, increasing susceptibility to infection and transplant-related mortality. We analyzed the longitudinal dynamics of gut microbiota in HSCT patients using Oxford Nanopore full-length 16S rRNA sequencing. Fecal samples from eight patients with hematologic malignancies were collected at three timepoints: pre-conditioning (D0), day 30 (D30), and day 100 (D100) post-HSCT (n=17 samples; expansion to ~39 samples and 15 total patients ongoing). The full-length 16S rRNA gene was sequenced using Oxford Nanopore MinION, taxonomically classified using Emu against the SILVA reference database, and analyzed after rarefaction normalization using standard diversity metrics and PERMANOVA. In this preliminary cohort, genus-level richness significantly decreased post-HSCT (Kruskal-Wallis p=0.034), by ~70% from baseline to day 100. Shannon diversity exhibited comparable reductions (about 44% decline), though statistical significance was limited by the small sample size. Principal coordinates analysis revealed temporal clustering tendencies along PC1, accounting for 29% of the variation. Taxonomic profiling demonstrated a pronounced shift toward pathobiont dominance, with opportunistic taxa capable of acquiring and disseminating antimicrobial resistance determinants (*Enterococcus*, *Klebsiella*) increasing from ~19% to 58% by day 30, accompanied by depletion of beneficial commensals, particularly *Blautia* and *Faecalibacterium*. Individual trajectories varied, with ~50% of patients exhibiting >70% loss in richness, a pattern associated with heightened susceptibility to infection, bacteremia, and adverse post-transplant outcomes, while others showed more moderate or biphasic patterns. No major batch effects were observed. These findings indicate pronounced HSCT-associated dysbiosis characterized by substantial loss of diversity and pathobiont dominance. The marked inter-patient heterogeneity suggests opportunities for personalized microbiome monitoring. Continued cohort expansion will enhance statistical power to identify predictive microbiome-based biomarkers of post-HSCT complications, underscoring the value of longitudinal microbiome monitoring as a potential adjunct to post-transplant patient management.

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Inulin supplementation partially restores gut dysbiosis in diet-induced obese mouse models: a systematic review and meta-analysis of metagenomics data

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Obesity and its metabolic comorbidities are rising worldwide, posing a major public-health challenge. Mounting evidence implicates the gut microbiome in obesity pathophysiology, impacting nutrient absorption, lipid and glucose metabolism, and inflammatory responses. Dysbiosis, disruption of microbial balance, is frequently observed in obesity, while diet, including prebiotics such as inulin, can modulate gut microbiome composition. In this context, we examined the effects of inulin, a soluble dietary fiber widely found in more than 36.000 plant species, in the restoration of the gut microbiome in obesity mouse models. Following the PRISMA guidelines, we conducted a systematic review and meta-analysis of next-generation 16S rRNA sequencing metagenomics data on diet-induced obese mouse models. We synthesized alpha-diversity metrics (richness, evenness, within-sample complexity) using weighted means (WM) and standardized mean differences (SMD), and compared relative abundances of dominant bacterial taxa using mean differences (MD). We found that obesity was characterized by a modest, metric-dependent impact on within-sample diversity (SMD: Shannon 0.59, Simpson −0.19, ACE −0.24,Chao1 1.30). Inulin treatment did not significantly alter alpha diversity of obese mice. In contrast, obesity resulted in pronounced taxonomic shifts, with large mean differences in the two dominant phyla (≈ −38 % for Firmicutes and ≈ +37% for Bacteroidetes). Notably, inulin partially reversed these changes; WM of Firmicutes were 43.98% in control, 66.19% in obesity, and 38.96% in obesity+inulin mice, while WM of Bacteroidetes were 53.27%, 33.17%, and 47.43%, respectively. In conclusion, our findings highlight the beneficial role of inulin supplementation which partially re-balances the gut microbiome in diet-induced obesity at the taxonomic level, while effects on alpha diversity are limited. The effect of inulin on lower-abundance taxa and functional pathways warrants further investigation.



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Targeting collagens through plant-derived microRNAs (xenomiRs)

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Beyond serving as a structural scaffold, the extracellular matrix (ECM) actively regulates a wide range of cellular processes in both physiological and pathological contexts. Among its components, collagens—comprising 28 family members—constitute the predominant structural proteins, with type I being most abundant across tissues and type II enriched in cartilage. These fibrillar collagens integrate with other ECM molecules to establish the tissue's three-dimensional architecture and mechanical properties. Within the tumor microenvironment, aberrant collagen remodeling and deposition contribute to matrix stiffening, tumor progression, therapeutic resistance, and immune modulation, positioning collagens as key biomarkers and potential therapeutic targets. RNA, one of the four fundamental macromolecules of life, plays a central role in gene regulation and expression. In plants, small non-coding RNAs are particularly abundant, with small interfering RNAs (siRNAs) and 21–24 nucleotide microRNAs (miRNAs) representing the most prevalent classes. miRNAs act as pivotal regulators of gene expression and developmental processes. Emerging evidence further suggests that plant-derived dietary miRNAs (xenomiRs) may persist after ingestion and modulate gene expression across kingdoms, potentially influencing human physiology. In this study, we investigated plant-derived xenomiRs from species native to the Evia region of Greece for their potential to target human collagen genes. We describe a comprehensive workflow encompassing RNA extraction and purification, small RNA library preparation and circularization, high-throughput sequencing, and *in silico* prediction of plant miRNA targets. This integrated framework enables the systematic identification of xenomiRs with putative roles in collagen regulation and cancer biology.

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Computational and functional analysis exploring conformational folding of protein variants in Alzheimer's disease

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Predicting the three-dimensional structure of proteins directly from their amino acid sequence remains a challenge in biomedical research. Recent advances in AI-power algorithms provide a transformative effect in solving this problem. AlphaFold, a deep learning tool that produced groundbreaking results in CASP14, has since been used for highly accurate structural predictions even when there are very little to no known homologous structures. In this study, a set of proteins and mutated protein variants that are ranked pathogenic for Alzheimer's disease was performed and their structural simulations were evaluated using the ColabFold implementation of AlphaFold which utilizes MMseqs2 algorithm to quickly figure multiple sequence alignments. Our findings suggest that potent conformations could be accurately modeled at a molecular level in their wild-type form while compared regions were correctly folded and the estimated metrics were almost exclusively in the "high accuracy prediction" range. Our study also demonstrated that only a few residues with sufficiently strong destabilization effects can exert detectable structural (backbone) deviation from the wild-type model.

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Phosphorylation-dependent recruitment of HIF-1α at specific genomic loci drives triacylglycerol biosynthesis in hypoxic cancer cells

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Adaptation to low oxygen entails changes in transcriptional regulation, which is fundamentally important in various diseases, including cancer. Hypoxia Inducible Factor-1 (HIF-1) coordinates hypoxic response and allows cancer cells to endure the hypoxic tumor microenvironment. ERK1/2-mediated phosphorylation of the oxygen-sensitive HIF-1α isoform at Ser641/643 serves a dual function. First, this modification promotes HIF-1α nuclear accumulation by masking a nuclear export signal (NES) and preventing its interaction with nuclear exportin CRM1[1]. Moreover, it enhances the interaction between HIF-1α and nucleophosmin (NPM1), an association essential for the productive binding of HIF-1 to gene-target promoters[2]. To further understand the role of HIF-1α phosphorylation, different GFP-HIF-1α forms that mimic or impair ERK1/2 phosphorylation at HIF-1α Ser641/643 residues (termed SE or SA respectively) or carrying Ser641/643Ala mutations and a mutated NES (IA/SA) were stably expressed in a HeLa *HIF1A*$^{-/-}$ cell line created by CRISPR/Cas9. Cell death assays revealed that only cells expressing the wild-type or the mutant phospho-mimetic SE form of HIF-1α could survive under hypoxia. Furthermore, total proteome analysis of these cells revealed a correlation between the phosphorylation status of HIF-1α and the genes selected for activation. Chromatin immunoprecipitation experiments followed by ChIP-sequencing showed that all HIF-1α forms could associate with transcriptionally active chromatin under hypoxia, albeit with different efficiency. Subsequently, we identified a specific gene cluster preferably occupied by the phospho-mimetic SE form. Furthermore, RIME (Rapid Immunoprecipitation Mass spectrometry of Endogenous proteins) analysis revealed that the phospho-mimetic form associates with chromatin components facilitating interactions between distal regulatory elements with promoters. By combining the proteomic and ChIP-seq results with phenotypic experiments, we discovered that HIF-1α phosphorylation could greatly enhance triacylglycerol biosynthesis in hypoxic cells. Taken together, our results shine further light on the mechanism by which ERK1/2-mediated phosphorylation controls the HIF-1α/chromatin interactome and drives HIF-1 gene target selection and lipid metabolism under hypoxia.

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Physical interaction of HIF-1α with the cohesin complex stimulates transcription of metabolic genes

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Lower-than-normal oxygen levels, termed hypoxia, characterize pathological conditions such as cancer. Hypoxia causes metabolic and transcriptional reprogramming, which is facilitated by a small family of transcriptional regulators called HIFs (Hypoxia Inducible Factors). HIFs exist in three main isoforms and consist of one alpha and one beta subunit. The oxygen regulated HIF-1α isoform is subjected to many post-translational modifications, including its direct phosphorylation, which affect its stability, subcellular localization and interactions with other proteins[1]. Recent mass spectrometry results of proteins co-immunoprecipitated with HIF-1α, showed that HIF-1α potentially interacts with the SMC1A subunit of the cohesin complex, which plays a key role in defining topologically associated domains (TADs) on chromatin[2]. The association between HIF-1α and SMC1A was verified by HIF-1α immunoprecipitation and immunoblotting analysis. To map the HIF-1α domain mediating this interaction, SMC1A cDNA was cloned into PGEX-4T-1 bacterial expression vector, GST-SMC1A was overexpressed in bacteria, purified and subjected to *in vitro* pull-down assays with purified HIF-1α fragments. The results revealed a direct interaction of the HIF-1α C-terminal domain with SMC1A. Furthermore, CLIP (Cross-linking and immunoprecipitation) experiments from extracts of cells stably expressing different phospho-deficient or phospho-mimetic HIF-1α forms showed that HIF-1α phosphorylation by ERK1/2 enhances the HIF-1α/SMC1A interaction, a result that was also confirmed by immunofluorescence microscopy. Finally, transcriptional activity experiments after SMC1A silencing suggest that the interaction between HIF-1α and SMC1A stimulates the transcription of a subset of HIF-1 target genes directly involved in triacylglycerol biosynthesis. Taken together, our results have revealed a novel HIF-1α interaction with SMC1A, which facilitates the expression of important metabolic enzymes under hypoxia. This may be mediated by cohesin-dependent chromatin remodeling, a hypothesis which is currently under investigation.

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Identification of Prognostic Serum Biomarkers in Non-Small Cell Lung Cancer Using Omics Technologies

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Background/Aim: Lung cancer remains the leading cause of cancer-related mortality worldwide, underscoring the need for innovative diagnostic and prognostic tools. This study aimed to investigate serum profiles in two distinct non-small cell lung cancer (NSCLC) patient groups in the course of their treatment –those presenting with positive response to treatment and those who had disease progression– aiming to identify omics signatures relevant to treatment response.

Materials and Methods: A total of 55 crude serum samples of NSCLC patients diagnosed at the "Sotiria" General Hospital, were prospectively collected in the course of patient treatment (n=8 12 months – positive response group, n=6 12months, disease progression group). Proteins were extracted using a modified filter-aided sample preparation (FASP) protocol, digested with trypsin, and analyzed by nano-liquid chromatography coupled with high-resolution Orbitrap mass spectrometry. Data were processed with Proteome Discoverer and R-based bioinformatics tools to identify and quantify differentially expressed proteins.

Results: A total of 3,820 proteins were identified across samples, with 13 showing statistically significant differential expression between patients with progressive disease and those with favorable prognosis. Gene Ontology enrichment analysis revealed biological functions related to immune response, coagulation cascades, and enzyme inhibition activity, consistent with literature evidence linking these proteins to lung cancer pathogenesis and progression.

Conclusion: A list of 13 serum proteins, including A2M, CP, C3, APOA1, and SERPINA1, emerge as potential non-invasive prognostic biomarkers. Their biological relevance and detectability in serum suggest strong translational potential for early prognosis and subsequent therapy adjustment for better NSCLC management.