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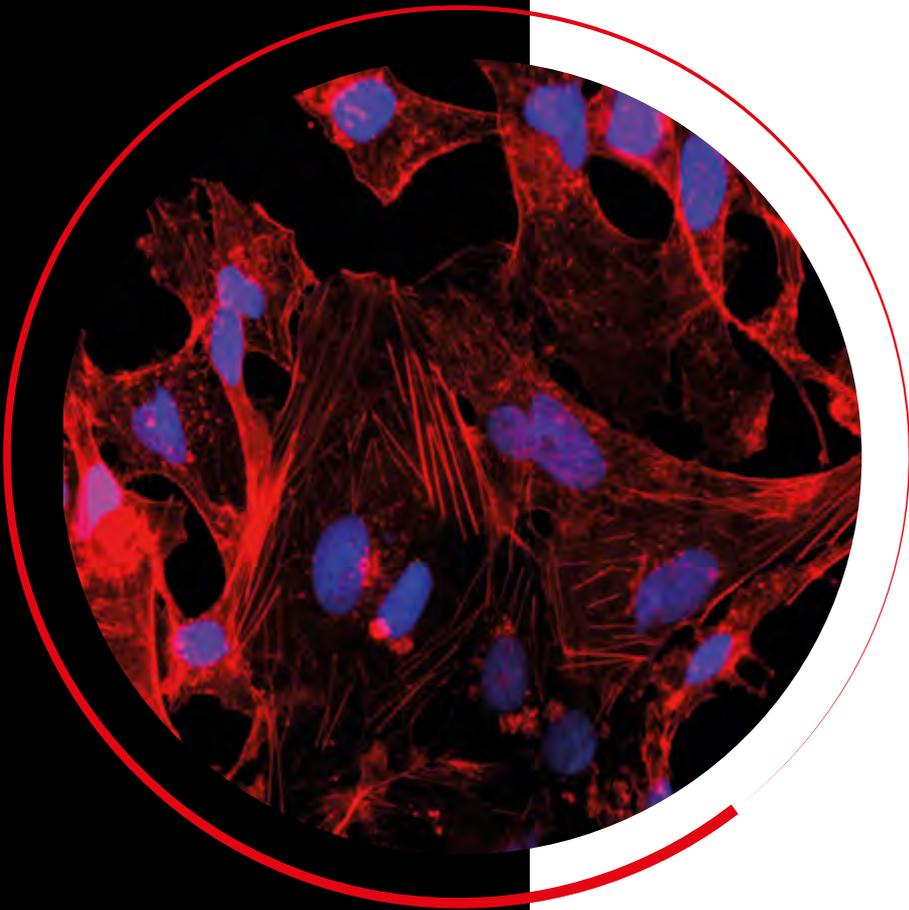
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ABSTRACTS BOOK

INVITED | PLENARY LECTURES



Gut bless you: Elucidating the role of probiotics in health and disease

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Probiotics are defined as microorganisms that confer health benefits to the host when administered in adequate amounts. Accumulating data suggest their ability to prevent or manage digestive disorders, such as Crohn's disease and ulcerative colitis, skin and bone diseases, even stress, depression and anxiety. Although, supplements and foodstuffs containing these microorganisms comprise a fast-expanding market, consumers and regulatory bodies are rather skeptical toward health claims on probiotics. In this talk, I will present work that is being conducted in my lab to systematically characterize the properties of novel probiotic strains, isolated from human hosts or fermented foodstuffs. In particular, we study their strain-specific ability to adhere to human cells and induce anti-proliferative effects, their antimicrobial potential and immunomodulatory capacity, using established *in vitro* and *in vivo* models, supported by robust bioinformatic analyses. In this context, we aim at deciphering the underlying cellular and molecular pathways involved in host-microbe crosstalk focusing on apoptotic and TLR-signaling. Finally, we are currently investigating the effect of probiotic supplementation on Immune Checkpoint Inhibitors (ICIs) response of lung cancer patients, in an ongoing clinical study. The holistic study of the impact of probiotics on the host, considering anthropometric parameters and gut microbiome composition can set the foundation for tailor-made, precision probiotic supplementation.

Mapping and Dating Extracellular matrix in Atherosclerosis

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Atherosclerotic cardiovascular disease is the largest underlying cause of death and disability in the world, leading to the formation of plaques in the innermost part of the arterial wall, which can ultimately rupture or erode, causing thrombosis and decreasing partly or fully the blood flow. Our group was the first to show in living humans using carbon-14 dating that plaque development occurs silently over 10 or more years before the actual clinical event. Therefore, there is a clear knowledge gap consisting of how to detect on time the plaques that will cause the clinical events, like heart attacks and strokes, and ideally treat them so that they do not rupture nor erode. These vulnerable plaques usually have a large lipid and necrotic core, are rich in inflammatory cells and are covered by a thin fibrous cap with a unique extracellular matrix signature. Possible approaches for early detection of vulnerable plaques include dating plaque components formation, circulating biomarkers and better understanding of the pathophysiologic mechanisms of the disease. Our observations support the notion that a specific extracellular matrix signature (glycosaminoglycans, proteoglycans) may be associated with a vulnerable plaque phenotype and a higher risk to suffer from future events.



Conformational landscape of protein kinases in physiology and disease

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Protein kinases regulate almost every aspect of cellular function. Changes in the expression, localization in the cell, mutations or chromosomal rearrangements of kinases can cause a number of cancers and other diseases. Cancer 'driver' mutations occur very frequently in kinase genes. In fact, the kinase domain is the domain most frequently encoded by cancer genes. Tremendous progress has been made in understanding the structure, function, and mechanisms of regulation of protein kinases. However, it has proved challenging to monitor these transitions and structurally characterize the manifold of conformational states inherently populated by a kinase. In the absence of such information, the mechanisms underpinning the response of kinases to physiological and pathological processes remain poorly understood. I will discuss how we structurally and energetically dissect the mechanisms underpinning the function and operation of a number of important protein kinases. We elucidate regulatory and drug-resistance mechanisms as well as how key structural elements and motifs control the activation/inhibition processes in kinases.

Neutrophils and NETs in physiology and disease

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Although neutrophils are the most abundant leukocyte in blood and the main cellular population of acute inflammation, they have been neglected by the scientific interest up to recent years where they just had their "renaissance". Their "functional simplicity" was challenged in 2004 when a new neutrophilic mechanism was described, Neutrophil Extracellular Traps - NETs, that redefined our perspective on their immune responses. Moreover, there are recent evidence on various neutrophilic subpopulations and even on circulation of immature forms of neutrophils with different functions. The fact that NETs have different protein constitution depending their inflammatory environment, even bearing disease-specific proteins, and the presence of various neutrophils populations with distinctively different functions, shed light on how these "simple" cells can influence the physiology but also the pathophysiology of such range of disorders (autoinflammation/autoimmune, thrombosis, cancer, tissue remodeling/fibrosis).



Molecular mechanisms of selective autophagy

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Autophagy is an evolutionarily conserved catabolic process that involves the degradation of cytoplasmic material through the lysosomal pathway. It is a cellular response in nutrient starvation but it is also responsible for the removal of damaged proteins and organelles. Dysfunction in autophagy has been implicated in an increasing number of diseases from bacterial and viral infections to cancer and neurodegeneration. Sequestration and degradation of cytoplasmic material by autophagy is selective through receptor and adaptor proteins. We are using mammalian cells in vitro and the fruit fly *Drosophila melanogaster*, as a genetically modifiable model organism to investigate the mechanisms of autophagy and selective autophagy in the context of the physiology of the cell, the system and the living organism. I will present some work from my lab related to molecular mechanisms of autophagy focusing on organellophagy.

Elucidating long non-coding RNA function in the human genome

Evgenia Ntini

IMBB-FORTH, Crete Greece

The vast majority of the human genome is transcribed into non-coding RNA, during 'pervasive' transcription. This creates transcriptional noise, which is under tight control by dedicated regulatory mechanisms. Still, some long non-coding RNAs (lncRNAs) harness mRNA-processing mechanisms, and are transcribed from enhancer-like regions and the anchor points of chromosomal loops. This suggests some functional regulatory potential. By implementing bioinformatics for the systematic analysis of transcriptomics data, we can characterize active enhancers in the human genome, and unravel their association with lncRNAs in conferring regulation of target gene expression. Molecular features that underlie sub-nuclear enrichment of lncRNAs, and their association to chromatin and RNA-binding proteins, can be identified via machine learning. Thus, bioinformatics and systematic approaches help reveal hidden layers of complexity in the human genome, and uncover novel roles of lncRNAs in regulation of gene expression.

Basic pharmacology and translational opportunities for hydrogen sulfide

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Over the last two decades, hydrogen sulfide (H_2S) has emerged as an important endogenous gasotransmitter in mammalian cells and tissues. Similar to the previously characterized gasotransmitters nitric oxide and carbon monoxide, H_2S is produced by the body by enzymatic reactions and regulates a host of physiological and pathophysiological processes in various cells and tissues. H_2S production and H_2S tissue levels are decreased in a number of conditions (e.g. diabetes mellitus and aging) and are increased in other states (e.g. various forms of inflammation and critical illness). Multiple approaches have been identified for the therapeutic exploitation of H_2S , either based on H_2S donation or inhibition of H_2S biosynthesis. H_2S donation can be achieved through the inhalation of H_2S gas, and/or the parenteral or enteral administration of various formulations of fast-releasing H_2S donors (salts of H_2S such as NaHS and Na₂S), or slow-releasing H_2S donors (GYY4137 being the prototypical compound). On the side of pharmacological inhibition of H_2S synthesis, there are small molecule compounds targeting each of the three H_2S -producing enzymes CBS, CSE and 3-MST. During the presentation, examples of the biological activities, along with translational efforts using H_2S donors and H_2S biosynthesis inhibitors in cardiovascular disease, obesity and cancer will be highlighted.

Novel genetic therapeutic approaches for beta-hemoglobinopathies

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Hemoglobinopathies are the most common inherited disorders in the world. Patients with severe beta-thalassemia major are currently kept alive through life long transfusions and iron chelation regimens while sickle cell disease patients receive preventive and symptomatic therapy for acute pain crises. The only curative approach for both disorders is the allogeneic bone marrow transplantation. However, only about 30% of patients have access to a suitable donor and the procedure is mostly suited for younger patients. Lentiviral mediated gene therapy using autologous cells is currently in clinical trials of patients with β hemoglobinopathies. Such treatment harbors several safety concerns (insertional mutagenesis, toxic myeloablative conditioning), together with low efficacy of corrected hematopoietic stem cells and risk of silencing long-term. Recent advances in genome-editing techniques have made it possible to target and modify any desired DNA sequence by employing programmable nucleases. The development of these custom designed genome and epigenome editing tools offers a novel therapeutic alternative for these patients. In this talk we will go over the different editing modules (zinc finger nucleases, TALE nucleases and CRISPR/Cas9) and their derivatives and their application in β -hemoglobinopathies.

Bacterial retrons a new type of phage defense systems

Athanasios Typas

European Molecular Biology Laboratory, Germany

The foundations of molecular biology have been established in the mid of the 20th century by studying bacteriophages, the viruses of bacteria. Restriction-modification systems and CRISPR, tools that have propelled genetic engineering, are systems that bacteria use to defend against phage attack. Yet, only recently we have started to understand how extensive and diverse are interactions between bacteria and phages. Myriads of bacterial immunity systems are being identified, many being the origins of eukaryotic innate immunity systems, and phages seem to have come up with even more ways to circumvent them. We have recently identified that the enigmatic bacterial retrons, the first prokaryotic elements discovered to encode a reverse transcriptase, act as phage defense systems. Bacterial retrons encode tripartite toxin-antitoxin systems, which use the complex of the reverse transcriptase with its DNA product both as antitoxin and as a sensor of phage protein activities. As response, they help the attacked cell to defend via abortive infection. Here, I will provide evidence on how these systems work and evolve to sense different phage activities, as well as how phages try to circumvent them.

Macromolecular crowding in eukaryotic cell culture

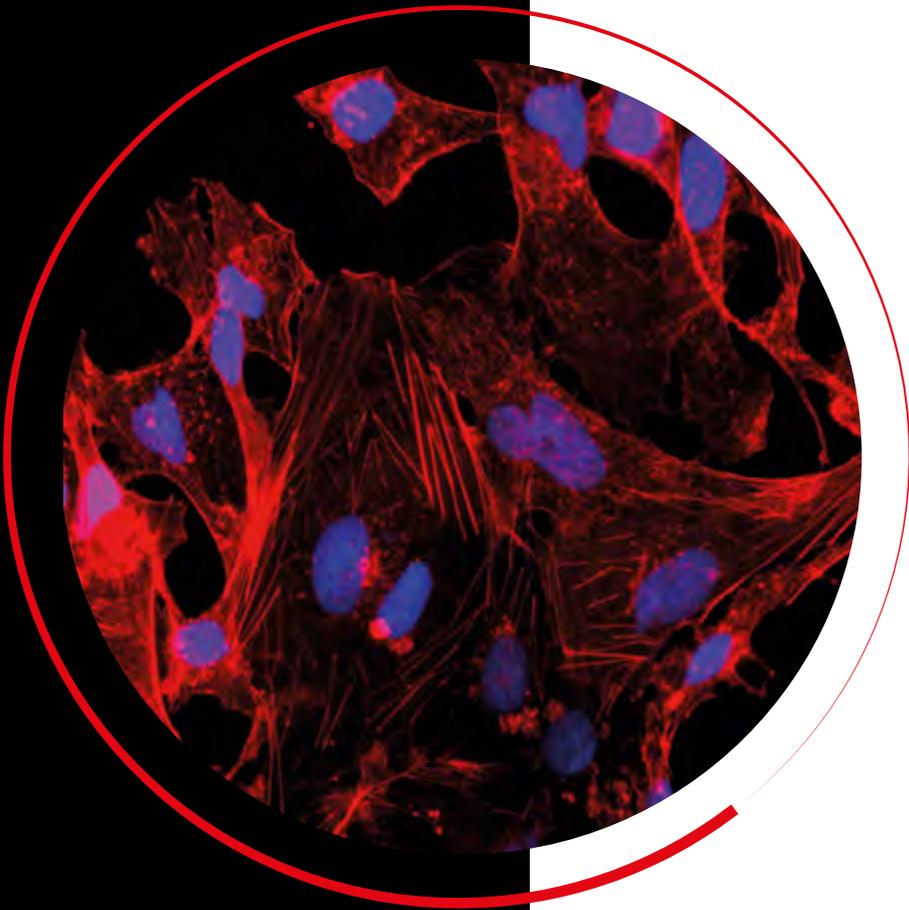
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In vivo, the extracellular space is heavily crowded by a diverse range of macromolecules. In contrast to nature's paradigm, traditional *in vitro* eukaryotic cell cultures are conducted in very dilute media. This talk will discuss the concept of macromolecular crowding, alone or in combination with other *in vitro* microenvironment modulators, in both permanently differentiated and mesenchymal stromal cell cultures.

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SHORT TALKS



SHORT TALKS 1 (ST1-9)

MOLECULAR & CELLULAR BASIS OF HUMAN DISEASES I

ST1

The role of replication stress in the activation of inflammatory response

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Accurate transmission of genetic information in daughter cells during cellular division is a fundamental procedure for the maintenance of genome integrity. Therefore, DNA replication underlies strict regulation since various agents often jeopardize genomic stability. Interestingly, cases of its dysfunction lead to development of aberrant DNA replication, generally known as replication stress, a major source of genomic instability that consists of either stalled and completely disrupted DNA replication or re-replication. Replication stress-induced genomic instability has been thoroughly proven to promote tumorigenesis and is recently considered to be implicated in the activation of innate immune response.

The replication licensing factor Cdt1 and its inhibitor Geminin are key regulators of DNA replication. Geminin binds and inhibits Cdt1 preventing relicensing of replication during the same cell cycle. Previous work of our research group has shown that alterations in expression levels of these proteins lead to re-replication, are linked to DNA damage and thereby, enhance murine lung and colorectal carcinogenesis. Here, we are investigating the link between the aberrant expression of Geminin and Cdt1 -representing our approach to replication stress- with inflammatory response activation. Towards this direction, we generated a colon cancer cell line HCT116 using an auxin-inducible degron system for the conditional depletion of Geminin. In this context, preliminary results of late stages of Geminin deletion reveal upregulation of interferon-stimulated genes as well as enhanced STAT1 phosphorylation both implying inflammatory pathway activation. In addition, we are genetically engineering a doxycycline-inducible HCT116 cell line to inhibit Cdt1 degradation and thus study the effects of Cdt1 overexpression in innate immune response. Among the long-term goals of the proposed research work is firstly to provide insight on the mechanism that activates inflammatory response in cases of genomic instability as well as to elucidate how inflammation determines cancer progression and finally to reveal molecular targets for anticancer therapy.

ST2

The WISP-1/MIF signalling axis in breast cancer cells

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Breast cancer is a highly heterogeneous disease that may exist in multiple subtypes, some of which still lack targeted and effective therapy. Therefore, a major challenge is to unravel the molecular mechanisms underlying the aggressive properties of breast cancer cells and bring to light novel therapeutic targets. WNT-inducible signaling pathway protein-1 (WISP-1) appears to be involved in various types of cancer, including breast cancer, in which it mainly exhibits an oncogenic role. Another mediator that seems to be involved in cancer development and progression is the macrophage migration inhibitory factor (MIF), a crucial pro-inflammatory cytokine and a multifunctional regulator. In this project, we are investigating the role of WISP-1/MIF axis in the metastatic potential of breast cancer cells as well as in specific constituents of the tumor microenvironment. To this aim, we treated MCF7 ERα+ breast cancer cells with WISP-1 and assessed the expression levels of MIF and its cellular receptor CD74. Further, we examined the mRNA expression of several matrix effectors, such as CD44, which also acts as a co-receptor for MIF, hyaluronan synthases (HASes), MMPs, heparanase, VEGF, EGFR and EMT markers. Moreover, we treated cells with WISP-1 in the presence of the Src kinases inhibitor PP2 and the MIF inhibitor ISO-1, to explore the role of Src kinases and MIF in WISP-1-mediated effects on the expression of the above effectors. Our results showed that WISP-1 potently induces the expression of MIF and affects the expression of specific matrix effectors with established roles in the promotion of cancer cell aggressive potential. Importantly, our results indicate that Src kinases and MIF are actively implicated in these processes. These findings demonstrate a critical role of WISP-1/MIF axis in breast cancer metastatic potential and suggest it as a potential therapeutic target.

ST3

Prolonged adrenergic stress induces mitochondrial alterations and primes the innate immune response in salivary gland epithelium

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Stress is a normal 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 autoimmunity; however, the molecular mechanisms are poorly understood¹.

Mitochondrial damage is connected to innate immunity since mitochondrial stress activates a series of events leading to type I interferon (IFN) response. Type I IFN signature is a hallmark of Sjögren's syndrome (SS) and other autoimmune diseases such as Systemic Lupus Erythematosus. SS 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 SS is mainly characterized by increased adrenergic signaling resembling chronic stress conditions. Thus, SS-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 SS-derived SGEC, as mitochondrial proteins show non-canonical response to it and induces mitochondrial stress as seen by aberrant mitochondrial morphology, loss of electron density and reduction of membrane potential, accompanied by the induction of interferon-beta 1 (IFN β 1) and increased expression of interferon-stimulated genes, such as OAS1 and OAS3. These data demonstrate that PAS compromises mitochondria machinery and primes innate immune responses in SS-derived SGEC.

ST4

The novel lentiviral vector IFN β /HF displays therapeutic efficacy as a gene therapy-based approach for multiple myeloma

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Multiple myeloma (MM) represents a malignant plasma cell disorder characterized by severe clinical manifestations and complications. In the context of developing novel therapeutic approaches for MM, we investigated the efficacy of an IFN β -expressing lentiviral vector, pseudotyped with the measles virus H and F glycoproteins (IFN β /HF) in myeloma cell lines and in MM patients' CD138⁺ cells.

Cytotoxicity was evaluated using CCK-8. Apoptosis and cell cycle were estimated by AnnexinV/7-AAD and PI staining, respectively. Expression of apoptotic genes was determined by qPCR and a Human Apoptosis Antibody Array. IFNs secretion and its paracrine action were determined by ELISA and Transwell[®] co-culture. Autophagy gene expression was assessed employing qPCR and confocal microscopy. Neutralizing antibody activity was determined by flow cytometry utilizing GFP/HF.

IFN β /HF exhibited a mean transduction efficiency of 29.5% (H929) and 23.1% (JN3), with mean VCN/cell of 1.6 and 1.2, respectively. IFN β /HF-transduction (MOI=1), led to a dramatic reduction of cell survival followed by a marked increase of apoptosis: 90.3% ($p \leq 0.001$) in H929, 74.1% ($p \leq 0.001$) in JN3, 91.82% ($p = 0.004$) in U266, and 92.59% ($p = 0.002$) in RPMI-8226 cells. Notably, the increase of Caspases 3 and 9 highlights the crucial role of the intrinsic apoptotic pathway in the IFN β -induced apoptosis, whereas a decrease of autophagy gene expression (e.g. Beclin-1, Atg4b, Atg5) was detected. After Transwell[®] co-culture, IFN β secreted by the transduced cells, was capable of significantly decreasing the viability of *untransduced* cells. Furthermore, IFN β /HF efficiently transduced primary cells, increasing IFN β and IFN γ secretion and decreasing cell survival, with a 51.8% increase of apoptosis ($p = 0.01$) and induction of cell cycle arrest. Since the antibody titer against measles virus is low, IFN β /HF can overcome the immunological barrier using a higher MOI.

These data document that IFN β /HF represents a promising therapeutic candidate for selective oncolytic action for MM.

ST5

CXCR4 and JUNB in circulating tumor cells isolated from non-small (NSCLC) and small cell lung cancer (SCLC) patients

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Recent studies have indicated that JUNB and CXCR4 contribute to metastasis in various cancer types. We have previously showed that JUNB and CXCR4 are overexpressed in circulating (CTCs) and disseminated tumor cells (DTCs) from breast cancer patients and are correlated with poor survival. In the current study we evaluated the expression of JUNB and CXCR4 in circulating tumor cells (CTCs) of lung cancer patients and examined the clinical significance of these molecules. For this purpose, ISET membranes from 30 patients with non-small cell lung cancer (NSCLC) and cytopins from 37 patients with small cell lung cancer (SCLC) were analyzed using confocal and VyCAP microscopy. Our results revealed that both JUNB and CXCR4 were expressed in the vast majority of lung cancer patients. Interestingly, the percentages of the phenotypic patterns differed between NSCLC and SCLC patients; the (CK+/JUNB+/CXCR4+) phenotype was present in 50% of NSCLC vs 71% of SCLC patients. Correspondingly, the (CK+/JUNB+/CXCR4-) was present in 44% vs 71%, the (CK+/JUNB-/CXCR4-) in 6% vs 71% and the (CK+/JUNB-/CXCR4-) phenotype in 38% vs 84%. Differences were also observed, regarding the average phenotypic percentages from the total number of CTCs in NSCLC and SCLC cases; the (CK+/JUNB+/CXCR4+) was present in 42% of NSCLC vs 19% of SCLC CTCs, the (CK+/JUNB+/CXCR4-) in 33% vs 27%, the (CK+/JUNB-/CXCR4-) in 6% vs 14% and the (CK+/JUNB-/CXCR4-) in 18% vs 41%. In NSCLC, the presence of ≥ 1 CTCs with the (CK+/JUNB+/CXCR4+) phenotype was associated with worse progression-free survival (PFS) ($p=0.007$, HR=5.21) while ≥ 2 CTCs with poorer overall survival (OS) ($p<0.001$, HR=2.16). In SCLC patients with extensive disease, the presence of ≥ 4 CXCR4-positive CTCs was associated with shorter OS ($p=0.041$, HR=5.01). In conclusion, JUNB and CXCR4 are overexpressed in CTCs from lung cancer patients and are associated with worse survival, suggesting their importance as potentially prognostic biomarkers for lung cancer patients.

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ST6

The implication of ORF3a protein of SARS-CoV-2 in lung (A549) and intestinal (Caco-2) epithelial cells

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The gene of ORF3a SARS-CoV-2 protein has been cloned into the pT7-IRES-His-C DNA plasmid vector to get its transcript for a closer approach of its entrance in the host cell and for mimicking protein's behavior as a constituent of the whole virus genome. The protein ORF3a which is the largest among the accessory proteins of SARS-CoV-2, consists of 274 amino acids (~33 kDa) and contributes to both the formation of new virions and virus pathogenicity. The produced mRNA has been encapsulated and transferred into the host cells for translation, by using the jetMESSENGER reagent/KIT. Its biological role was investigated in lung (A549) and intestinal (Caco-2) epithelial cells, as components of the main tissues affected during SARS-CoV-2 infection. The protein's involvement in the extrinsic pathway of apoptosis has been verified by qPCR experiments which unambiguously indicated the clear expression of the apoptotic factors *BID*, *Caspase 8*, *BIM*, *BAX*, *BAK*, *BAD*, *Bcl-2*, *RIPK-1* and *CHOP*. The expected inflammatory response of lung and intestinal epithelial cells after their transfection with ORF3a-mRNA was confirmed by the expression of inflammatory factors *IL1 β* , *IL18*, *IL6*, *IL8*, *ISG15* and *nF- κ B*. Studies regarding the subcellular localization of ORF3a-GFP after its cloning into pEGFP-N1 vector and by using the above cell lines indicated its preference in the nucleoplasm and cytoplasm, leaving the nucleoli area vacant (Caco-2) or in the cytoplasm, leaving empty nuclei (A549), respectively. These findings strongly suggest the interaction of ORF3a with the transcriptional factor HCFC1 which is mainly localized in the nucleoplasm. When the HCFC1R is highly expressed, its "counterpart" HCFC1 is transported into the cytoplasm and this event supports our above results ensued by the confocal microscopy. Taken together, the interaction of ORF3a with HCFC1 and its transport into the nucleoplasm, as potent cellular step for protein's implication in cancer, will be further investigated.

ST7

Novel oxLDL markers in chronic kidney disease patients on maintenance hemodialysis

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Chronic kidney disease is commonly associated with cardiovascular diseases (CVDs), a proven oxidative stress (OS)-induced causative factor of which is oxidized LDL (oxLDL). The present study seeks possible association of patients with end-stage kidney disease on maintenance hemodialysis (CKD-5d) with CVD risk development, by introducing, for the first time, specific oxidative modifications on the main LDL protein/lipid components as more valid clinical markers for oxLDL status quantitative assessment. LDL from 61 CKD-5d patients and 40 healthy controls is isolated/fractionated, by new methodologies, into its main lipid (cholesteryl esters, triglycerides, free cholesterol, phospholipids) and protein (apoB100) sub-fractions, the oxidative modifications of which are quantitatively assessed by lipid hydroperoxides (-OOH) in LDL lipid sub-fractions and malondialdehyde and dityrosines in apoB100. Free cholesterol-/triglyceride-OOH markers are significantly elevated in CKD-5d versus control, and are unaffected by single hemodialysis session duration, underlying medical conditions, sex, age, years of hemodialysis, and medication (not even statins). Notably, LDL-C levels are inversely correlated with increased free cholesterol-OOH, while HDL-C levels are inversely correlated with both increased markers. The new oxLDL clinical markers besides their OS-association with CKD-5d could become more reliable tools for assessing CVDs risk than the currently employed LDL-C/HDL-C.

ST8

LSD1-driven mechanisms in HCV-induced HCC

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Hepatocellular carcinoma (HCC), the most common type of liver cancer, largely occurs after chronic infection with the Hepatitis C Virus (HCV), while elimination of HCV by novel antivirals does not necessarily protect against development of HCV-related HCC. HCV induces epigenetic changes to the host in order to complete its life cycle, leading to metabolic dysfunction and malignant transformation. Our aim was to investigate the putative role of Lysine-specific demethylase 1 (LSD1), an epigenetic factor found overexpressed in HCC biopsies, in HCV infection and the facilitation of HCV-orchestrated oncogenic mechanisms.

LSD1 was shown to play a negative role in the establishment of HCV infection, since inhibition of LSD1, followed by HCV infection *in vitro*, caused higher rates of viral replication in hepatoma cells. At the same time, replication of HCV was found to be inhibited in LSD1-overexpressing hepatoma cells infected with HCV. Electroporation of the full length HCV genome and replication-competent subgenomic replicon in the presence of LSD1, partially restored HCV replication compared to control cells, suggesting that HCV might be inhibited by LSD1 during the steps of entry and replication. Furthermore, the use of replication-defective subgenomic replicon ruled out any effects of LSD1 on HCV translation. HCV-Core and -NS5A proteins were shown to fine-tune endogenous LSD1 expression levels throughout infection. Nevertheless, LSD1 increase across HCV replication cycles during long-term infection was of great concern, due to its role in HCC establishment and progression. Extracellular Matrix Metalloproteinase Inducer (EMMPRIN), known for its role in invasion and metastasis through matrix metalloproteinase (MMP) induction, was found activated in the presence of LSD1. Furthermore, secreted MMP-2 and -9 were increased, suggesting that LSD1 might contribute to metastatic HCV-driven HCC.

ST9

Autophagy activation can partially rescue proteasome dysfunction-mediated cardiac toxicity

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Protein quality control maintains proteome homeodynamics (proteostasis) and is critical for cellular functionality and viability especially for post-mitotic cells like cardiomyocytes, which are constantly exposed to proteotoxic, metabolic and mechanical stress. Proteostasis is assured by an integrate system called proteostasis network (PN), major components of which are the main degradation pathways i.e., the autophagy-lysosome and the ubiquitin proteasome pathways. Aberrant activation of the proteostasis mechanisms is found in advanced tumours and thus their inhibition provides promising anti-cancer therapies. We report that pharmacologically- or genetically- induced proteasome dysfunction in flies' (*Drosophila melanogaster*) heart, leads to increased proteome instability and disrupted mitostasis, resulting in perturbation in cardiac functionality, systemic toxicity, and reduced longevity. These phenotypes were partially rescued by either heart targeted- or by dietary restriction-mediated autophagy enhancement. Supportively, Metformin or Rapamycin (autophagy activators) administration mitigated the toxic effects caused by targeted proteasome malfunction in heart by restoring proteome instability and mitochondrial functionality. These data represent a relevant preclinical insight for alleviating cardiovascular complications due to proteasome impairment.

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SHORT TALKS 2 (ST10-18)

STEM CELLS, TISSUE MORPHOGENESIS & REGENERATION

CELLULAR AGEING

ST10

A cross-species strategy to identify new molecular players of the inflammatory response that promote heart regeneration

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The inability of adult human heart to regenerate damaged cardiac tissue leads to adverse remodelling events including fibrosis and promotes heart failure. These events have been linked with extensive and persisting inflammation. Nevertheless, characteristics of the inflammatory response including the maturity of resident macrophages or the activation status of infiltrating cells may differentially influence cardiomyocytes and cardiac fibroblasts, and thus regeneration. Unravelling crucial parameters of such interactions in appropriate biological systems should confer decisive intervention potential in a serious health problem.

In the past years we have studied cellular and molecular players regulating the progress of heart failure in mouse and heart regeneration in zebrafish, and we propose here to combine the systems to elucidate these interactions. Particularly, we are developing an approach to identify factors secreted by innate immune cells that affect cardiac regeneration. Based on *in vivo* and *ex vivo* systems and by using a cross-species platform we are assessing the effect of differentially activated immunocytes on cardiomyocyte proliferation and evaluate their pro- or anti-regeneration potency.

ST11

Synthesis and characterization of safe, biomimetic scaffolds with drastic peptides for bone and cartilage engineering

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Defects of the bone and cartilage affect all ages worldwide and research is focusing on the creation of safe biomaterials that regenerate the damaged tissue from autologous stem cells. In this project, biocompatible biomimetic scaffolds that combine the structure and mechanical properties of human elastin, silk fibroin and mussel-foot adhesive proteins were synthesized genetically. These biomaterials were produced as polypeptides with repetitive building blocks and crosslinked to form networks in the form of hydrogels, which contain cell-attachment peptides and drastic peptides from either BMP-2 for osteogenesis or TGF- β 1 for chondrogenesis. The elasticity and viscosity values of the crosslinked scaffolds were 3-4 orders of magnitude higher than in non-crosslinked biomaterials and they formed stable networks. SEM imaging revealed a porous surface with interconnected pores, while MTT cytotoxicity assays showed that growth of human dental pulp stem cells (hDPSCs) was optimal on 1 mg/mL of the scaffolds. Expression of osteogenic marker genes *ALP*, *RUNX2*, *Osteocalcin*, *COL1A1*, *BMPR1A* and *BMPR2* was upregulated 5-12 times in cells that were cultured on scaffold without BMP-2 peptide and 13-38 times in cells that were cultured on scaffold with the BMP-2 peptide, compared to untreated hDPSCs. The phospho-Erk/Erk ratio was higher in cultured cells on both scaffolds, indicating the induction of osteogenic signaling. To evaluate the effect of gut microbiota metabolites onto cells that grow in the presence of the biomaterial, Caco-2 colon cancer epithelial cells were cultured with scaffold and with/without culture supernatant of three anaerobic bacterial strains for up to 48 h. Cellular metabolites involved in β -oxidation of fatty acids and in biosynthesis of purines and DNA were increased after 24 h but decreased dramatically after 48 h, which may indicate that the scaffold offers partial protection against bacterial metabolites that cause overproliferation of colon cancer cells.

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ST12

Loss of SNAI1 induces cellular plasticity in invasive triple-negative breast cancer cells

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The transcription factor SNAI1 mediates epithelial-mesenchymal transition, fibroblast activation and controls inter-tissue migration. High SNAI1 expression characterizes metastatic triple-negative breast carcinomas, and its knockout by CRISPR/Cas9 uncovered an epithelio- mesenchymal phenotype accompanied by reduced signaling by the cytokine TGF- β . The SNAI1 knockout cells exhibited plasticity in differentiation, drifting towards the luminal phenotype, gained stemness potential and could differentiate into acinar mammospheres in 3D culture. Loss of SNAI1 de-repressed the transcription factor FOXA1, a pioneering factor of mammary luminal progenitors. FOXA1 induced a specific gene program, including the androgen receptor (AR). Inhibiting AR via a specific antagonist regenerated the basal phenotype and blocked acinar differentiation. Thus, loss of SNAI1 in the context of triple- negative breast carcinoma cells promotes an intermediary luminal progenitor phenotype that gains differentiation plasticity based on the dual transcriptional action of FOXA1 and AR. This function of SNAI1 provides means to separate cell invasiveness from progenitor cell de- differentiation as independent cellular programs.

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ST13

Deciphering epithelial-mesenchymal plasticity driven phenotypic heterogeneity in pancreatic cancer utilizing advanced organoid models

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Pancreatic ductal adenocarcinoma (PDAC) is characterized by the vigorous desmoplastic reactions and the pronounced inter and intra-tumoral heterogeneity resulting in increased chemoresistance and high mortality rates. While organotypic cultures have been widely used in the past decade to functionalize PDAC biology, these models fail to capture the morphological phenotypic diversity found in PDAC.

Here, using an advanced branching PDAC organoid model system, we aimed to generate a phenotypic organoid landscape and reveal molecular and phenotypic treatment-specific vulnerabilities. To this end, we generated single-cell derived PDAC organoids by culturing tumor cells inside 3D floating collagen gels from distinct molecular PDAC subtypes, namely classical and basal-like. Morphologically, epithelial organoids resemble the classical tumour architecture with thick branches, tubular end buds and a seamless lumen connecting the organoid body, while mesenchymal organoids grow as invasive star-like structures resembling anaplastic carcinomas. Branched PDAC organoids retain their transcriptional subtype and establish distinct phenotypes based on the parental PDAC subtype. Notably, we identified the existence of multiple distinct organoid phenotypes derived from individual PDAC cells derived from primary cell lines of the *Pdx1 cre;LSL-Kras^{G12D}* (KC) mouse model. To create phenotypic PDAC organoid landscape, we developed a novel methodology termed PHeMap (PDAC Heterogeneity Phenotype Mapping) employing deep convolutional neuronal networks to perform phenotypic classifications, revealing 8 major morphological clusters based on their distinct EMT status. In order to decipher how this diversity is achieved, we performed single-cell RNA profiling of parental tumor cells. To functionalize intra-tumor heterogeneity using our model system, we characterized distinct organoid phenotypes on a molecular level and performed multi-modal treatments to identify phenotype-specific vulnerabilities.

With our organoid assay we are able to capture pre-existing tumour cell heterogeneity determined by the EMT transition state of the cell-of-origin and find therapeutic vulnerabilities targeting cellular and phenotypic plasticity.

ST14

Metabolomics as a sensitive monitoring tool for CAR-T cell manufacturing process standardization

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T-cells expressing a synthetic chimeric antigen receptor (CAR-T) are a revolutionary novel treatment in hematology and oncology. Conventional CAR-T therapy has not been standardized yet and remains a complicated process, involving complex logistics from centralized manufacturing facilities, inflexible manufacturing and clinical use schemes that disregard patient and cell characteristics, thus limiting patient access and therapeutic outcome. The EC Horizon 2020 AIDPATH project will apply top-notch AI technology to integrate patient-specific data and biomarkers in CAR-T therapy and apply flexible manufacturing schemes to obtain CAR-T cell products with optimal fitness and anti-tumor potency. A major aspect of the project is the standardization of the various modules of the CAR-T manufacturing process, proposing quality control markers at major steps of the procedure.

Metabolomics has been proven as a sensitive monitoring tool of bioprocess consistency, providing a holistic perspective of the cellular metabolic physiology. In this way, the effect that certain stages of a multi-step process may have on cellular quality could be investigated and the particular stage(s) could be appropriately tuned. In addition, quality control markers and the accepted operation window for each step of the manufacturing process could be implied. This information will improve our knowledge about the CAR-T manufacturing process and contribute towards the establishment of quality control regulations. FORTH/ICE-HT is the metabolomics partner in AIDPATH. We are to compare various bioreactor setups currently used in the CAR-T process and various media. This involves the standardization of the sample collection and handling protocols according to the bioreactor type. We will show examples of such standardization for two bioreactor types. Moreover, we are to acquire the metabolic profile of CAR-T cells generated from patients in a clinical trial to complement the currently measured biomedical data, based on which the suitability of the CAR-T cells for the patients is decided.

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ST15

Conserved elements of post transcriptional regulation mechanisms in stress protection of *Drosophila*'s adult progenitor cells

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In *Drosophila*, most larval cells are polyploid and they die at the metamorphic stage. In contrast, Adult Progenitor Cells (APCs) survive throughout the developmental process and give rise to adult structures. These cells are specified during embryonic development, they undergo several mitotic divisions during larval stages, remain diploid, and finally proceed into their terminal differentiation during the pupal stages. Both APCs and larval tissue cells are exposed to the same nutritional and hormonal cues, thereby suggesting that unique molecular components act within the APCs to differentially regulate the effect of external and intrinsic stimuli in their unique setting.

We have shown that the *headcase* (*hdc*) gene, that was originally identified by its specific expression in *Drosophila* APCs, is one of the components that these cells use to survive, grow and terminally differentiate. Hdc acts at a systemic level, controlling the hormonal response during development, while in APCs it modulates the effects of the dTOR pathway, acts as a tumor suppressor and is implicated in the control of the Unfolded Protein Response.

Hdc is the founding member of a group of homolog proteins identified from *C. elegans* to humans. In humans, its homolog, termed HECA, has been found associated with different kinds of cancers but its function has not yet been identified and its role remains controversial. We provide evidence that both Hdc and HECA proteins interact with a conserved group of molecular elements that are known to control the RNA metabolic cycle, protect cells against stressful stimuli and form part of the post transcriptional mechanisms of gene expression control.

ST16

In vivo generation of allogeneic hearts in chimeric mice

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Many people worldwide are in need of heart transplants. Organs generated from the patient's own cells would not only solve the problem of transplant availability, but would also bypass the complication of incompatibility and tissue rejection by the host immune system. Induced Pluripotent Stem Cells (iPSC) hold great promise for regenerative medicine. Although considerable progress has already been made concerning the use of iPSCs in cell therapies, generation of whole organs has so far met with little success. Our lab is developing an *in vivo* method to make allogeneic hearts in chimeric mice, composed of host and donor cells. We have succeeded in generating a chimeric embryo made up of host and donor cells, with a heart consisting exclusively of donor cells. We have also designed a strategy to exclude donor cells from the chimeric body and produce a chimera with a donor-derived heart in a host-derived body. Our ultimate goal, is to transfer this technology and generate in big animals (such as pigs), human hearts for transplantation, from the patients' own cells.

ST17

In vivo and in vitro comparative analyses of Neural Stem Cell's function of adult brain, between laboratory and wild populations of *Mus musculus domesticus*

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In the postnatal rodent and human brain neural stem and progenitor cells (NSPCs) cluster within stem cell niches contributing to odour recognition, learning, memory and myelination. Based on experimental work, using the lab-mouse, numerous factors have been identified as regulators of NSPCs in their niches, including stress, exercise and neuronal activity; however, no significant clinical breakthrough has been made so far. In this work, we compare the activity of lab-mouse versus wild-rodent NSPCs, both in situ (in their natural microenvironment) and in vitro. The project builds on pilot work indicating that specific pools of NSPCs in the lab-mouse brain remain in a “tamed”, hypo-active, status, as the activity and density of the respective NSPCs in the brain of wild rodents (that face continuous stress, nutritional variations and maintain higher physical activity) are significantly higher. We use lab-mice as well as wild animals and we generate quantitative histological measurements and in vitro assessments of the properties of NSPCs, for the first time in the same animal, showing that limitations do exist, as far as laboratory mice are concerned. The conclusion of this work aims to assess the limitations of using lab animals in translational biomedical research and to validate the use of lab animals when studying the effects of molecules or interventions targeting NSPCs.

ST18

Sustained Nrf2 overexpression-mediated diabetic phenotypes can be partially attenuated by restoring insulin/insulin-like signaling

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Modulation of insulin/insulin-like growth factors signaling (IIS) is associated with altered nutritional and/or metabolic states. *Drosophila* encodes eight insulin like peptides (Ilps), whose activity is regulated by a group of secreted factors, including Ecdysone-inducible gene L2 (ImpL2) that by binding to ImpL2 blind its activity. We recently reported that *cncC* (*cncC/Nrf2*), the fly ortholog of mammalian Nrf2, is a positive transcriptional regulator of *ImpL2*, as part of a build-in negative feedback loop aiming to suppress aberrant *cncC/Nrf2* activity, which (among others) leads to diabetes type I-like phenotypes. Here, we extend these studies in the fly model by assaying the functional implication of *ImpL2* in *cncC/Nrf2* overexpression (*cncC^{OE}*)-mediated metabolic deregulation. We found that *ImpL2* knockdown suppressed *cncC^{OE}*-induced insulin resistance, reverted energetic shortage and tissue wasting phenotypes and it largely rescued flies' health-/life-span. Further, pharmacological treatment of *cncC^{OE}* flies with the anti-diabetic drug Metformin restored (dose-dependently) normal IIS, attenuated tissue atrophy and extended flies' longevity. Our findings provide additional mechanistic details on how prolonged stress signaling by otherwise cytoprotective short-lived stress sensors (i.e., *cncC/Nrf2*) perturbs IIS leading to diabetic phenotypes; they also highlight the therapeutic effects of Metformin in the treatment of pro-diabetic prolonged stress signaling.

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FEATURED TALK

ST19

COVID-19 pathophysiology and anti-SARS-CoV-2 vaccines mode of action

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COVID-19 is marked by increased virus infection rates due to the wide expression of ACE2 [binding site of the Spike (S) viral protein] and a number of infection enabling proteases (e.g., TMPRSS2 and CTSB/L) in tissues of the respiratory and gastrointestinal tract. In the elderly and in the presence of certain pre-existing comorbidities infection may lead to uncontrolled inflammatory immune responses which drive hyper-cytokemia, aggressive inflammation and (due to broad organotropism of SARS-CoV-2) collateral tissue damage and systemic failure likely because of (among others) systemically imbalanced ACE/ANGII/AT1R and ACE2/ANG(1-7)/MASR axes signaling. Vaccination is a major tool for mitigating the COVID-19 pandemic and mRNA vaccines are central to the ongoing vaccination campaign that is undoubtedly saving thousands of lives. However, adverse effects (AEs) following vaccination have been noted which may relate to a pro-inflammatory action of the employed lipid nanoparticles or the delivered mRNA (i.e., vaccines formulation), as well as to the unique nature, expression pattern, binding profile, and pro-inflammatory effects of the produced antigens (i.e., S protein and/or its subunits-peptide fragments) in human tissues/organs. Our on-going multidisciplinary research (to be reported) on both topics has revealed significant molecular insights to both COVID-19 pathophysiology, as well as to vaccines induced immune responses and AEs. Increased knowledge on these issues will guarantee safety, maintain trust, and direct health policies regarding e.g., the anti-COVID-19 vaccination campaign.

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SHORT TALKS 3 (ST20-31)

MOLECULAR & CELLULAR BASIS OF HUMAN DISEASES II

ST20

Investigation of Body Mass Index as a factor for Type 2 Diabetic Kidney disease in a greek cohort

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Diabetic kidney disease (DKD) is a severe complication of diabetes and is the major cause of end-stage renal disease (ESRD). Although tight glycemic control can reduce the rates of DKD and ESRD, a substantial number of patients develop DKD despite adequate glycemic control, while others with chronic severe hyperglycemia are relatively spared. Obesity has been posited as an independent risk factor for both diabetic and nondiabetic renal disease, with Body Mass Index (BMI) commonly used as a proxy measure for obesity. We investigate the interplay between BMI and DKD by conducting a GWAS study on a discovery cohort composed of 143 DKD cases and 207 controls. DNA was extracted from whole blood samples and genotyped on the Illumina PsychChip array. After quality control, we performed genotype imputation using the HRC reference panel on the Sanger Imputation Server. We performed sex-stratified association tests using linear regression on BMI and DKD status as variables, and secondary phenotypic characteristics, such as age and smoking status as covariates. We identify rs7725250 (p:7.669e-07) residing near PPAP2A, the intergenic rs560807731 (p: 2.274e-08), and rs1322550 (p: 8.465e-08) residing near IMPG1 and MYO6. These results contribute towards the elucidation of the polygenic genetic landscape of DKD. Future directions involve investigating how these genes associate with one another and with the environment to contribute to the interplay between DKD and BMI.

ST21

The mRNA expression of apoptosis- and autophagy-related genes in prostate cancer cells is modulated upon treatment with bortezomib and carfilzomib

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Proteasome inhibitors constitute one of the most important drug classes applied for the treatment of hematological malignancies, and they have received increasing interest for the treatment of solid tumors as well. The mechanisms of action of proteasome inhibitors that lead to cell death are diverse and affect many cellular pathways. Herein, the expression levels of apoptosis and autophagy-related genes after treatment of prostate cancer cells with bortezomib and carfilzomib were studied by cell proliferation, reverse transcription, and PCR techniques. Firstly, we determined the optimal seeding concentration of 0.75×10^5 , 1.0×10^5 , and 0.85×10^4 cells/mL for DU 145, PC-3 and LNCaP cells, respectively, where cell culture concentration would augment exponentially until 72 hours. Next, cells were treated with either bortezomib or carfilzomib at three-time points (24, 48, and 72h), to determine the inhibition concentration (IC_{50}) in 72 hours. The IC_{50} for bortezomib was 5nM for DU 145 and LNCaP, and 10nM for PC-3. The IC_{50} for carfilzomib was 50nM for DU 145, 30nM for LNCaP, and 60nM for PC-3. Overall, in LNCaP and PC3 cell lines after treatment with bortezomib or carfilzomib, we observed a significant increase in the expression levels of pro-apoptotic genes: *BAK1*, *BAX*, and *TP53*, and in the levels of autophagy-related genes: *SQSTM1* (*p62*), *BECN1* (*Beclin 1*), and *PIK3C3*. In contrast, we demonstrated a decrease in the expression levels of the anti-apoptotic gene *BCL2*. Regarding the DU 145 cell line, after treatment with bortezomib, we observed an increase in the expression levels of pro-apoptotic and autophagy-related genes. However, these findings were not validated after treatment with carfilzomib. In conclusion, bortezomib was less cytotoxic and seems to be more effective than carfilzomib in the treatment of prostate cancer cell lines. Lastly, our data show that proteasome inhibition activates more than one form of cell death in prostate cancer cells.

ST22

Impaired hepatic glucose metabolism and liver- α -cell axis in mice with liver-specific ablation of the Hepatocyte Nuclear Factor 4a (*Hnf4a*) gene

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Hnf4a gene ablation in mouse liver causes hepatic steatosis, perturbs HDL structure and function and affects many pathways and genes related to glucose metabolism. Our aim was to investigate the role of liver HNF4A in glucose homeostasis using mice with liver specific ablation of *Hnf4a* (*Alb-Cre;Hnf4a^{fl/fl}*, H4LivKO) and their littermate controls (*Hnf4a^{fl/fl}*). H4LivKO mice presented lower blood levels of fasting glucose, improved glucose tolerance, increased serum lactate levels and reduced response to glucagon challenge compared to their control littermates. Insulin signaling in the liver was reduced despite the increase in serum insulin levels. H4LivKO mice showed altered expression of genes involved in glycolysis, gluconeogenesis and glycogen metabolism in the liver. The expression of the gene encoding the glucagon receptor (*Gcgr*) was markedly reduced in H4LivKO liver and chromatin immunoprecipitation assays revealed specific and strong binding of HNF4A to the *Gcgr* promoter. H4LivKO mice presented increased amino acid concentration in the serum, α -cell hyperplasia and dramatic increase in glucagon levels suggesting an impairment of the liver- α -cell axis. Glucose administration in the drinking water of H4LivKO mice resulted in an impressive extension of survival. The expression of several genes related to non-alcoholic fatty liver disease progression to more severe liver pathologies, including *Mcp1*, *Gdf15*, *Igfbp1* and *Hmox1*, was increased in H4LivKO mice as early as 6 weeks of age and this increased expression was sustained until the endpoint of the study. Our results reveal a novel role of liver HNF4A in controlling blood glucose levels via regulation of glucagon signaling. In combination with the steatotic phenotype, our study suggests that H4LivKO mice could serve as a valuable model for studying glucose homeostasis in the context of non-alcoholic fatty liver disease.

ST23

Identification of alternatively spliced, circular transcripts (circRNAs) of the *PRMT1* gene in breast cancer cell lines, using targeted nanopore sequencing

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Circular RNAs (circRNAs) constitute a class of RNA molecules that are formed through back-splicing. In breast cancer, circRNAs are proven to be implicated in major cellular processes and affect tumor onset and progression. Although methylation by *PRMT1* has been well documented to affect breast cancer cell properties, the effect of circular transcripts deriving from this gene has not been elucidated yet. The purpose of this study was to identify novel *PRMT1* circRNAs in breast cancer cells and to untangle the unique alternative splicing events that occur during back-splicing. In order to achieve this, total RNA was extracted from 11 breast cancer cell lines of distinct characteristics and molecular subtypes, and a normal human breast epithelial cell line, followed by reverse transcription with random hexamer primers. Next, first- and second-round PCRs were performed using gene-specific divergent primers, in order to selectively amplify *PRMT1* cDNAs. Then, long-read third-generation sequencing with nanopore technology was performed in the MinION Mk1C platform with the Flongle adapter. Extensive bioinformatics analysis was conducted, using publicly available tools and programs such as minimap2 and samtools, as well as *in-house* developed algorithms. From this pipeline, more than 100 novel circRNAs were identified, which consist of both exonic and intronic regions of the *PRMT1* gene. Moreover, extensive splicing events were also revealed. In most cases, the exons that form the back-splice junction were truncated in most circRNAs, and multiple splice sites were found for each exon. Extensions of the known exon boundaries were abundant as well. Intriguingly, poly(A) stretches were also present in several circRNA structures, and this finding raises new questions regarding the biogenesis mechanisms of circRNAs. In conclusion, this study revealed the complete sequence of novel circRNAs of the *PRMT1* gene, comprising distinct back-splice junctions and probably having different molecular properties.

ST24

PD-L1, CTLA-4, GLU and VIM Expression in Triple Negative Breast Cancer Patients' CTCs and their Clinical Impact

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Triple Negative Breast Cancer (TNBC) is the most aggressive subtype of breast cancer plus there are limited targeted therapies available for these patients, leading in an unmet need for new biomarkers. The aim of the present study was to investigate the expression of programmed death-ligand 1 (PD-L1), cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), detyrosinated α -tubulin (GLU) and vimentin (VIM) in Circulating Tumor Cells (CTCs) of TNBC patients and assess their relationship with severity of disease and clinical outcome. Fifty-two TNBC patients were enrolled: 35 early and 17 metastatic. PD-L1, CTLA-4, GLU and VIM expression was identified by immunofluorescence and VyCAP platform. The expression of GLU⁺VIM⁺CK⁺ phenotype was higher (47%) in metastatic TNBC compared to early TNBC patients (20%) ($p = 0.043$). In addition, incidence of the PD-L1⁺CD45⁻CK⁺ phenotype was higher (86%) in metastatic compared to early TNBC patients (69%) and the CTLA-4⁺CD45⁻CK⁺ phenotype was also higher (47%) in metastatic compared to early TNBC patients (26%). Among patients, significant correlation was found between PD-L1⁺CD45⁻CK⁺ and CTLA-4⁺CD45⁻CK⁺ phenotypes (Spearman test, $p = 0.029$), whereas significant correlation was also indicated between PD-L1⁺CD45⁻CK⁺ and GLU⁺VIM⁺CK⁺ phenotypes (Spearman test, $p = 0.013$). Both phenotypes GLU⁺VIM⁺CK⁺ (log rank $p = 0.045$) and PD-L1⁺CD45⁻CK⁺ (log rank $p < 0.001$) were associated with shorter overall survival (OS) of TNBC patients. To the best of our knowledge, this is the first study focusing simultaneously on PD-L1, CTLA-4, GLU and VIM expression in CTCs of TNBC patients. Our findings demonstrate that PD-L1, CTLA-4, GLU and VIM constitute significant biomarkers in TNBC. They are associated with disease progression and patients' outcome, providing an interesting tool for stratifying patients that could benefit from novel combination of targeted therapies.

ST25

Evaluation of a Polygenic Risk Score model based on the protein interactions of Psoriasis susceptibility loci

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Linkage studies have uncovered fifteen different genomic loci that contribute to the pathogenesis of Psoriasis, known as Psoriasis Susceptibility (PSORS) loci, with the respective proteins participating in immune and skin-related pathways. Interestingly, not all these loci have been statistically associated from the largest Genome-Wide Association Study (GWAS), such as PSORS4 and PSORS8¹. Aberrant interactions between proteins (Protein-Protein interactions; PPIs) of the PSORS loci at a pathological state perturbate the individual's homeostasis and lead to the inflammatory phenotype. In this study we propose and develop a novel PPI-based Polygenic Risk Score (PRS) approach for Psoriasis, comparing its discriminatory ability with rigorous, statistical approaches. We filtered the UK BioBank² cohort for European, unrelated clinical Psoriasis cases and grouped with randomly selected healthy participants using an at least 1:4 ratio over cases as to maximize statistical power. PPIs were constructed via the implementation of two major meta-databases of protein interactions: PICKLE 3.0³ and InnateDB⁴. Single-Nucleotide polymorphisms (SNPs) of the latest GWAS summary statistics¹ were mapped to the GRCh37/19 reference genome and filtered for their chromosomal location based on the identified PSORS-interacting genes. To begin with, our novel PPI-PRS approach was compared to a PRS constructed with various P-value thresholds as well as a combined model of both PPI and P-thresholding approaches. 360.710 independent variants were mapped in the 1575 non-overlapping, PSORS-interacting genes. The PPI-PRS analysis depicted a predictive ability, as measured by the AUC of 0.6463, a marginal difference compared to the $P \leq 0.1$ approach (AUC: 0.6566), incorporating 1.015.916 SNPs. Additionally, the combined PRS model, despite increasing the independent SNPs ($n=1.336.41$), displayed a marginal difference to our PPI approach (AUC: 0.6578). In conclusion, we present a novel, biological-driven approach for the computation of PRS via the protein interactions of associated genes, shrinking therefore the abundance of the variants utilized in the calculation of PRS.

¹ Tsoi LC, Stuart PE, Tian C, et al. Large scale meta-analysis characterizes genetic architecture for common psoriasis associated variants. *Nat Commun.* 2017;8:15382.

² Sudlow C, Gallacher J, Allen N, et al. UK biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. *PLoS Med.* 2015;12(3):e1001779.

³ Dimitrakopoulos GN, Klapa MI, Moschonas NK. PICKLE 3.0: Enriching the human Meta-database with the mouse protein interactome extended via mouse-human orthology. *Bioinformatics.* 2020;37(1):145-146.

⁴ Breuer K, Foroushani AK, Laird MR, et al. InnateDB: systems biology of innate immunity and beyond--recent updates and continuing curation. *Nucleic Acids Res.* 2013;41(Database issue):D1228-D1233.

ST26

Unraveling the role of RANKL in breast cancer-derived bone metastasis

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Receptor activator of nuclear factor- κ B ligand (RANKL) has been previously reported to drive the process of osteoclast differentiation and maturation, playing, thus, a critical role in bone resorption and osteoporosis. Within the last decade, increasing evidence suggests that RANKL and its cognate receptor RANK are associated not only with bone resorption but also with the development and progression of breast cancer. In the present study, we investigated the involvement of RANKL in breast-cancer related metastases using both *in vitro* and *in vivo* techniques. *In vitro* stimulation of E0771 murine mammary cancer cells with recombinant RANKL resulted in increased expression of key molecules downstream of RANKL signaling, as well as of markers associated with epithelial to mesenchymal transition (EMT) and metastasis. In addition, we established a mouse model of bone metastasis in transgenic mice that overexpress human RANKL (TgRANKL) by injecting E0771 cells, systemically. Since the E0771 cell line is stably transduced with the firefly luciferase gene, we were able to monitor skeletal metastasis incidence and progression by *in vivo* bioluminescence imaging. Our imaging results revealed that TgRANKL mice developed bone metastasis earlier than their wild-type (WT) littermates. Histological analysis confirmed severe bone metastasis at both femurs and the tibiae of TgRANKL mice within 3 weeks post E0771 cell injections. In contrast, most WT mice did not develop signs of bone metastasis during this period. Apart from a higher tumor burden, TgRANKL mice also displayed extensive bone osteolysis, compared to control WT mice. Interestingly, prophylactic treatment of E0771-injected TgRANKL mice with Denosumab, a monoclonal antibody against human RANKL, prevented bone metastasis throughout the study period. Collectively, we have established a preclinical model of breast cancer-derived bone metastasis that is driven by RANKL, providing a unique system for understanding the underlying molecular mechanisms and the evaluation of novel therapies against skeletal metastasis.

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ST27

West Nile virus NS1 protein invades in innate immunity

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West Nile virus (WNV) is a neurotropic mosquito-borne virus, belonging to the genus *Flavivirus*, family *Flaviviridae*. To this date, there is no WNV vaccine that has been approved for use in humans, neither a virus-specific inhibitor. WNV NS1 protein is a multifunctional protein which acts as a cofactor for viral replication and assembly in the form of an intracellular dimer. A secreted, hexameric form of WNV NS1 protein has been shown to disrupt the innate immunity in cell culture by blocking important molecular pathways, such as TLR signalling and interferon signalling pathways. Moreover, it inhibits the activation of factors critical to the outcome of immune responses, such as NF- κ B, IRF3 and IL-6. In addition, it is associated with disease progression and severity, since it interacts with several proteins of the complement system and induces increased permeability of brain endothelial cells. In this study, we utilised a novel mouse model developed in our lab, which expresses WNV NS1 protein under cd11b promoter in myeloid cells, in order to mimic the first step of WNV infection after the mosquito bite. Bone marrow-derived macrophages expressing WNV NS1 protein presented alterations in expression profile of genes associated with interferon signalling. In particular, WNV NS1 expression resulted in the downregulation of major innate immunity genes, such as *Stat2*, *Irf3*, *Irf7*, *Isg15* and *Isg20*. The cd11b/WNV NS1 mouse model offers the opportunity for further WNV biological and pharmacological testing, while setting a platform for safer experimentation towards a WNV vaccine, by exploiting the capabilities of NS1 protein *in vivo*.

ST28

NR5A2 as a novel drug target in non-small cell lung cancer

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Lung cancer is one of the most frequently diagnosed malignancies with poor overall prognosis and high mortality rates worldwide. About 85% of incidences exhibit non-small cell lung cancer (NSCLC), mainly comprising adenocarcinoma (ADC) subtype. These clinical findings highlight the need for new insights into pharmaceutical targets and combination therapies. To this end, here, we identify NR5A2/LRH1, a druggable nuclear receptor, as a negative regulator of lung cancer progression. In particular, our metanalysis of clinical data from publicly available databases support a correlation between high NR5A2 expression levels and survival of lung cancer patients. Consistently, we experimentally show that NR5A2 is sufficient to suppress the proliferation of non-small lung cancer cell lines *in vitro* and *in vivo*. The antiproliferative effect is possibly mediated by the transcriptional induction of negative cell cycle regulator CDKN1A (encoding for p21^{cip1}) and the simultaneous downregulation of G1-S transition inducer CCND1 (encoding for cycle D1). Moreover, NR5A2 overexpression also inhibits cancer cell migration *in vitro*. Most importantly, a well-established agonist of NR5A2, dilauroyl phosphatidylcholine (DLPC), is able to recapitulate the antiproliferative action of NR5A2 in non-small lung cancer cell lines. Furthermore, gene expression analysis reveals an additional effect of NR5A2 on important metabolism related genes. These observations suggest a tumour suppressive role of NR5A2 in NSCLC. They also provide a preclinical proof of concept for its use as a potential pharmaceutical target for the treatment of lung cancer.

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ST29

Cold atmospheric plasma suppresses the growth of breast cancer cells through regulation of extracellular matrix effectors

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Breast cancer exists in multiple subtypes, some of which still lack targeted and effective therapy. Cold atmospheric plasma (CAP) is an emerging anti-cancer treatment modality. CAP is an ionized gas where the ions are close to room temperature and contains electrons, charged particles, reactive oxygen, and nitrogen species (ROS/RNS). The objective of this project is to investigate a CAP-based therapy for breast cancer. To this aim, the effect of CAP on the viability of breast cancer cells of different ER status and metastatic potential were examined by following three experimental approaches; direct treatment (where CAP was directly applied to cell cultures), indirect treatment (where CAP-treated media were transferred to cells) and medium change treatment (where CAP was directly applied to cells followed by immediate replacement of the treated medium by fresh). The effect of CAP treatment on breast cancer cell viability in the absence or presence of ROS scavengers with different specificity revealed the prominent role of ROS, in particular H₂O₂, in the observed cytotoxicity of CAP. The removal of the CAP-treated medium from the cells (medium change approach) abolished the cytotoxic effect indicating the prominent role of CAP-derived ROS in this process. Further, CAP treatment induced intense morphological changes and apoptosis (involving the mitochondrial pathway) in both ER⁺ and ER⁻ cells. Importantly, our data showed that CAP treatment regulates the expression of specific matrix effectors in breast cancer cells, since the expression of CD44 protein (a major cancer stem cell marker and matrix receptor) was reduced, while the expressions of proteases and inflammatory mediators were differentially affected. The findings of the present study suggest that CAP suppresses breast cancer cell growth and regulates several effectors of the tumor microenvironment and thus it could represent an efficient therapeutic approach for distinct breast cancer subtypes.

ST30

Astrocytic calcium signaling as a neuroprotective mechanism against α -synuclein-induced inflammation

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α -Synuclein aggregation has been linked with sustained neuroinflammation in Parkinson's Disease (PD) aggravating neuronal degeneration. Even though α -synuclein can be phagocytosed by microglia and/or astrocytes, the molecular pathways that trigger and prolong inflammation in the PD brain remain unclear. Aberrant astrocytic calcium signaling has been linked with the pathogenesis of several neurodegenerative diseases and could contribute to the initiation or maintenance of neuroinflammation. In the present study we investigated the role of calcium signalling in α -synuclein-induced inflammation in vivo, using the human α -synuclein A53T transgenic mouse model, where the presence of α -synuclein oligomers is correlated with sustained inflammatory responses, such as significant elevations in the levels of endogenous antibodies and pro-inflammatory cytokines, in the absence of cell death. Similar results were obtained in post-mortem human brain samples of PD patients. 3D cell reconstruction and morphometric analysis of the GFAP⁺ astrocytes in the striatum revealed significant astrogliosis as suggested by the increased number and distinct morphological and transcriptional alterations in the A53T mice indicative of type A1 reactivity. Further analysis of the striatum of A53T mice using immunofluorescence and immunoblotting revealed an activation of the p38/MAPK pathway in microglia and an unconstrained stimulation of the NF- κ B pathway in astrocytes. We found that such activation results in the upregulation of astrocytic T-type Ca_v3.2 voltage gated Ca²⁺ channel causing significant alterations in astrocytic calcium influx. We believe that the elevation of Ca_v3.2 levels is, at least in part, a compensatory mechanism to protect neurons from oligomeric α -synuclein-induced inflammation by promoting axon regeneration.

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ST31

Revisiting the schizophrenia-Akt signaling link

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Schizophrenia is characterized by a complex interplay between genetic and environmental risk factors converging on prominent signaling pathways that orchestrate brain development. The Akt/GSK3 β /mTORC1 pathway has long been recognized as a point of convergence and etiological mechanism, but despite evidence suggesting its hypofunction, it is still not clear if this is already established during the first episode of psychosis (FEP). Here, we performed a systematic phosphorylation analysis of Akt, GSK3 β , and S6, a mTORC1 downstream target, in fresh peripheral blood mononuclear cells from drug-naive FEP patients and control subjects. We have also assessed Akt/GSK3 β /mTORC1 pathway activity after a 6-week treatment of patients with antipsychotic drugs. Our results suggest two distinct signaling endophenotypes in FEP patients. GSK3 β hypofunction exhibits a promiscuous association with psychopathology, and it is normalized after treatment, whereas mTORC1 hypofunction represents a stable state. Our study provides novel insight on the peripheral hypofunction of the Akt/GSK3 β /mTORC1 pathway and highlights mTORC1 activity as a prominent integrator of altered peripheral immune and metabolic states in FEP patients.

SHORT TALKS 4 (ST32-40)

FUNCTIONAL GENOMICS AND PROTEOMICS

DNA DAMAGE/REPAIR

ST32

Cardiomyocytes derived from differentiated embryonic stem cells as a model to study metabolism and nitric oxide signaling

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Recent advances in the field of embryonic stem (ESCs) cells have provided opportunities for better understanding the molecular basis of human diseases and the development of disease models to test novel therapeutics.

Cardiomyocytes derive 70% of the ATP molecules that are required for normal contractile, ionic and other important functions through the mitochondrial β -oxidation pathway (mFAO). Deficiencies of FAO pathway result in inadequate ATP production, metabolic imbalance, and cardiac dysfunction.

Our understanding regarding the regulation of β -oxidation under pathophysiological conditions has lagged due to the lack of experimental models that recapitulate human physiology and disease.

Contracting cardiomyocytes derived from differentiated ESCs represent an attractive experimental model. Herein, we performed proteomic characterization of ESCs-derived cardiomyocytes following the expression levels of Troponin-T and SIRPA as markers of cells maturation. Western blot analysis documented the presence of both proteins in lysates prepared from cells differentiated for 14 days. Proteins were not detected in undifferentiated cells. Immunostaining of fixed cells as well as flow cytometric analysis of live cells revealed that both troponin-T and SIRPA were expressed in 70% of the cells differentiated for 14 days. Moreover, the expression of carnitine palmitoyl-transferase-2 (CPT2), very long chain acyl-CoA dehydrogenase (VLCAD) and trifunctional protein A (TFPa), the three obligate partners for mFAO, was documented in mature cardiomyocytes. To start gaining insights regarding the regulation of β -oxidation by nitric oxide signaling we examined the S- nitrosylation status of the three enzymes. We and others have shown that S- nitrosylation, an NO-derived posttranslational modification, regulates protein function and coordinates metabolic activity^{1,2}. Chemoselective enrichment followed by western blot analysis revealed the presence of CPT2, VLCAD and TFPa in the bound fractions indicating the endogenous S-nitrosylation of these proteins.

In summary, ESCs-derived cardiomyocytes express key enzymes participating to mFAO. Our study provides initial evidence regarding the regulation of this pathway by NO-signaling.



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References:

- ¹ Doulias PT, Tenopoulou M, et al. (2013). Nitric oxide regulates mitochondrial fatty acid metabolism through reversible protein S-nitrosylation. *Science Sig.* 6: rs1
- ² Tenopoulou M, Doulias PT, et al. (2018). Oral nitrite restores age-dependent phenotypes in eNOS-null mice. *JCI Insight.* 3: e122

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ST33

Developing a proximity dependent biotinylation methodology for identifying transient interactors of Golgi-bypassing cargoes

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Newly synthesized transmembrane proteins, such as transporters and receptors, are made on ribosomes attached to the ER. Upon co-translational translocation into the membrane of the ER, these proteins concentrate at specialized regions called ER exit sites, where they pack into COPII secretory pre-vesicles, which eventually bud and fuse with the cis-Golgi. Following Golgi maturation, membrane proteins exit from the *trans*-Golgi network in clathrin coated vesicles directed to the PM¹. However, recent findings from our group, using the fungus *Aspergillus nidulans* as a model system, showed that several transmembrane cargoes follow an 'unconventional' sorting pathway that bypasses the Golgi^{2,3}. To investigate how distinct cargo-specific COPII subpopulations are generated and sorted to the PM by a Golgi-independent mechanism, we aimed at developing a methodology for the identification of transient interactions of Golgi-bypassing cargoes. While assessing stable interactions is more straightforward, capturing transient ones is extremely challenging, and thus no transient interactions of transporters and receptors have been identified by unbiased approaches. To this direction we designed a methodology that uses proximity-dependent biotinylation assays coupled with LC-MS/MS⁴, adapted for unravelling transient interactors of the UapA transporter, our most extensively studied Golgi-bypassing cargo. To distinguish cargo-specific interactions from abundant endogenously biotinylated proteins or unspecific interactions, we constructed several *A. nidulans* strains expressing distinct C-terminal fusions of TurboID-biotin ligase with wild-type or mutant versions of UapA affected in trafficking (e.g., UapA-TurboID, UapA-*mini*TurboID, UapA-DYDY/AAAA, UapA- Δ C-TurboID, UapA- Δ C-*mini*TurboID, TurboID, UapA-TurboID/ Δ artA) and used varying conditions of cargo induction and exogenous biotin administration. We will present our first results and envisioned improvements of this technique.

Indicative Bibliography:

¹ Gomez-Navarro, N. & Miller, E. J. *Cell Biol.* 215, 769–778 (2016).

² Dimou, S. et al. *EMBO Rep.* 21, (2020).

³ Dimou S, et al. *Front Cell Dev Biol.* 10:852028 (2022)

⁴ Branon TC, et al. *Nat Biotechnol* 36: 880–887 (2018)

ST34

Defining virus-carrier networks that shape the composition of mosquitoes' and biting midges' core virome of a local ecosystem

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Viruses transmitted via blood-feeding insects (arboviruses), such as West Nile virus, Zika virus, bluetongue virus and lumpy skin disease virus, pose a significant threat to public health and animal husbandry worldwide, potentially causing epidemics with considerable and multifaceted repercussions for the infected host organisms. A considerable increase of arthropod-borne diseases has been reported over the past few years and pest-control of those blood-feeding insects is a top priority to prevent future outbreaks of viruses. Mosquitoes and biting midges constitute major vectors of these emerging virus diseases. Significant efforts have been made to elucidate the core virome of these insects in their natural habitats, although the vast majority of metagenomics approaches has focused on specific or only a few species. However, in most ecosystems, multiple species may participate in virus emergence and circulation, while there is lack of understanding on the viruses-carrier/host network for both vector-borne and insect-specific viruses. In this study, the core viromes of 24 species of mosquitoes and 10 species of biting midges were defined, which were all field-collected from the diverse ecosystem of the Eastern Macedonia and Thrace region on northern Greece, an important path of various arboviruses. We identified 48 viruses, including 29 novel viruses according to the respective families' species demarcation criteria. Comparison of the viromes revealed a complex virus-carrier network in the ecosystem and novel relationships between mosquito genera and virus families, as most of the mosquito species had never been analysed in the past. The proposed study emphasized on the importance of a holistic approach regarding insect viromes in rich and diverse ecosystems. The stability of the core virome seemed to determine the composition of the total core virome in a local ecosystem as it is directly related to the population of the respective species.

ST35**Investigation of complex pharmacogenomic region haplotypes****Ilias Makrodimitris, Panagiota Kalliakmani, Christina Pertsali, Fotios Tsetsos****Department of Food Science and Nutrition, University of the Aegean, Greece*

A multitude of genes reside in structurally complex, poorly understood regions of the human genome. Especially in the case of pharmaceutically interesting genes, that number can reach up to a hundred. The repetitive and rapidly evolving nature of such loci hinders any investigative efforts, masking them from high-throughput analyses, effectively blinding state-of-the-art approaches. We are developing several novel methodologies to overcome these challenges. Here we present the application of those approaches on three genes, ADH1A, CYP3A5, and DPYD. We utilized data from the Human Structural Variation Consortium to investigate partially or fully unresolved regions, using Long Read Sequencing or Short Read Sequencing. We identify six common structural haplotypes per gene, leading to a resolution of 85-90% of the region, outperforming previous results by 30-40%, quantifying structural variation composition, as in inversions, deletions, insertions, segmental duplications and copy number variation. Our research aims to document the full extent of genetic variation in such loci in diverse human genomes, and to provide comprehensive analysis of this variation for utilization in high-throughput experiments.

ST36

Serum Proteome Signatures of Anti-SARS-CoV-2 Vaccinated Healthcare Workers in Greece Associated with Their Prior Infection Status

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Background/Aim: Over the course of the pandemic, proteomics, being in the frontline of anti-Covid-19 research, has massively contributed to the investigation of molecular pathogenic properties of the virus. However, data on the proteome on anti-SARS-CoV-2 vaccinated individuals remain scarce. This study aimed to identify the serum proteome characteristics of anti-SARS-CoV-2-vaccinated individuals who had previously contracted the virus and comparatively assess them against those of virus-naïve vaccine recipients.

Materials and Methods: Blood samples of n=252 individuals, out of whom n=35 had been previously infected, were collected in the "G. Gennimatas" General Hospital of Thessaloniki, from 1/4/2021 to 8/31/2021. All participants received the BNT162b2 mRNA Covid-19 vaccine (Pfizer/BioNTech). A label-free quantitative proteomics LC-MS/MS approach was undertaken and the identified proteins were analyzed using the GO (Gene Ontology) and KEGG (Kyoto Encyclopedia of Genes) databases as well as processed by bioinformatics tools. Titers of total RBD-specific IgGs against SARS-CoV-2 were also determined using the SARS-CoV-2 IgG II Quant assay.

Results: A total of 47 proteins were found to be significantly differentially expressed, the majority of which were down-regulated in sera of previously infected patients compared to virus-naïve controls. Several pathways were affected supporting the crucial role of the humoral immune response in the protection against SARS-CoV-2 infection provided by Covid-19 vaccination.

Conclusion: Our comprehensive proteome profiling analysis contributes novel knowledge of the mechanisms of immune response induced by anti-SARS-COV2 vaccination and identified protein signatures reflecting the immune status of vaccine recipients.

References:

Stamoula, E.; Sarantidi, E.; Dimakopoulos, V.; Ainaizoglou, A.; Dardalas, I.; Papazisis, G.; Kontopoulou, K.; Anagnostopoulos, A.K. Serum Proteome Signatures of Anti-SARS-CoV-2 Vaccinated Healthcare Workers in Greece Associated with Their Prior Infection Status. *Int. J. Mol. Sci.* 2022, 23, 10153. <https://doi.org/10.3390/ijms231710153>

Dimou, S.; Georgiou, X.; Sarantidi, E.; Diallinas, G.; Anagnostopoulos, A.K. Profile of Membrane Cargo Trafficking Proteins and Transporters Expressed under N Source Derepressing Conditions in *Aspergillus nidulans*. *J Fungi (Basel)* 2021, 7. <https://doi.org/10.3390/jof7070560>

ST37**Generation of Genetic Sexing Strains for the Mediterranean fruit fly
*Ceratitis capitata*****Antonia Spanomitrou^{*1}, Georgios Papadopoulos¹, Kostas Mathiopoulos¹**¹Department of Biochemistry and Biotechnology, University of Thessaly, Larissa, 41500, Greece

The Mediterranean fly (*Ceratitis Capitata*) is a sub-species derived from the family of Tephritidae and causes a significant amount of economical loss. At the moment its population is controlled with chemical pesticides with a lot of negative environmental consequences. The Sterile Insect Technique (SIT) is environmentally friendly, with which large insect quantities bred in lab are sterilized and consequently are released in the field. Sterile male insects mate with wild females which results in the birth of sterile eggs and thus in the reduction of the wild population. The separation of the male population from the female one before the release is quite important. The release of both gender population significantly reduces the efficiency of this technique and taken the fact that only males are necessary for this method to work into consideration and thus there is no reason for the development, radiation, transfer and release of the females which even though are sterile, still compete the rest of the females in mating and cause damage to crops with egg birth. Here, we present our study in the development of a genetic sexing strain for the Mediterranean fly, which is based in the introduction of temperature sensitive mutations. These mutations will be lethal to females just by shifting to higher temperatures than their rearing temperature. They will be designated with rational design and will be tested in computational models before their application by predicting their phenotype. The mutations with the desired lethal phenotype will be incorporated with the CRISPR/Cas9 methodology. This study will contribute in the enrichment of the SIT in the Mediterranean fruit fly and the species of Tephritidae in general and will target in their efficient and radical treatment.

ST38

Palaeoproteomics; method development and proteomics analysis on human bones

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Proteomics analysis based on mass spectrometry (MS) is a widely used scientific field, involving the large-scale investigation of the structure and function of a wide range of proteins and their post-translational modifications. Mass spectrometry-based proteomics enables the study of palaeoanthropology, bioarchaeology, archaeology and forensic sciences in general. It offers valuable information about proteins, protein pathways and their post-translational modifications, unlike common anthropology techniques or DNA analysis. Palaeoproteomics analysis has offered a variety of protocols on different kinds of samples (1), like art items, teeth and bones of various organisms, in order to answer evolutionary or dietary questions, for sex estimation and for dating samples.

The aim of this current study was to design and develop an MS-based proteomics methodology for the study of modern and ancient human samples, particularly bones, in order to create an auxiliary and complementary tool.

A total of 9 fractioned samples was used, 8 modern from the Cretan collection and 1 ancient sample from 3200 BCE. Sample selection, preparation, digestion, desalting and nano liquid chromatography-tandem mass spectrometry (nLC-MS/MS) analysis were conducted following an already established protocol with noteworthy modifications (2).

This study was successful in extracting, digesting, identifying and relatively quantifying proteins from human bone samples, as well as identifying unique proteins, which could be used in construction of an individual's biological profile. Sex-specific proteins were detected on several occasions, which makes proteomics analysis of bone samples a valuable tool with great potential in assisting identification of human remains when recovered incomplete and fragmentary. It is necessary to proceed to a further bioinformatics analysis, including other proteomes from bacteria or fungi, in order to identify possible contaminants related to taphonomy and environmental conditions. In addition, there is the need to extend our sample number and it is important to design a standard sampling and cleaning methodology.

Key words: human bones, bone proteomics, forensic anthropology, nLC-MS/MS, proteins

Bibliography

² Hendy J et al., (2018). A guide to ancient protein studies. *Nat Ecol Evol.*; 2(5): 791-799.

¹ Cappellini E, Jensen LJ, Szklarczyk D, et al. (2011). Proteomic analysis of a pleistocene mammoth femur reveals more than one hundred ancient bone proteins. *J Proteome Res.*; 11(2): 917-926.

ST39

Post-replicative replication stress and suppression of telomere transcription in the Alternative Lengthening of Telomeres in neoplasia, generate long single-stranded telomeric G- and C-rich DNA overhangs

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Despite their heterochromatic nature, telomeres are transcribed into G-rich, long non-coding RNAs termed TERRA. TERRA play an important role in telomere structure and regulate cellular and organismal proliferation capacity through the two known pathways of telomere maintenance (telomerase activity or Alternative Lengthening of Telomeres-ALT). To investigate the role of TERRA in the interplay between telomeric one-, or two-ended DNA double strand break repair in telomerase-independent telomere length maintenance in neoplasia, we examined the effects of ATR inhibition and the depletion of classical homologous recombination (c-HR) and Break Induced Replication factors (BIR) such as BRCA1, BARD1, and RAD52 respectively, as well as the helicase UPF1 that is involved in genomic DNA-RNA hybrid formation and TERRA regulation. In addition, we utilized a telomeric TALEs-based model of conditional suppression of TERRA in a subset of chromosome termini in human cancer cells relying upon ALT to sustain neoplastic cell proliferation, as well as an experimental setup of replicative and post-replicative replication stress that allows to examine the newly described post-replicative homology mediated transcription coupled DNA repair at the genome and at the telomeres. Our results reveal a differential behavior of genomic versus telomeric DNA repair in ALT cells at the G2/M interval of the cell cycle. Furthermore, we uncover for the first time, replicative stress driven, microscopically visible telomeric G- and C-rich DNA overhangs providing a mechanistic explanation on their generation, their protection from exonucleolytic insults, and their repair. Our results broaden our understanding on telomeric DNA damage responses holding promise for the development of novel therapeutic strategies against incurable cancers depending on ALT.

References:

- ¹ Feretzaki M, Pospisilova M, Valador Fernandes R, Lunardi T, Krejci L, Lingner J. RAD51-dependent recruitment of TERRA lncRNA to telomeres through R-loops. *Nature*. 2020 Nov;587(7833):303-308.
- ² Silva B, Arora R, Bione S, Azzalin CM. TERRA transcription destabilizes telomere integrity to initiate break-induced replication in human ALT cells. *Nat Commun*. 2021 Jun 18;12(1):3760.
- ³ Ngo GHP, Grimstead JW, Baird DM. UPF1 promotes the formation of R loops to stimulate DNA double-strand break repair. *Nat Commun*. 2021 Jun 22;12(1):3849.

ST40

Sequence Determinants of CRISPR/Cas9 Scissile Profile for Precise Genome Editing

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CRISPR/Cas9 is a powerful DNA targeting platform for precise genome editing, holding immense potential for successful gene therapy of various genetic diseases. To fulfill this role, it is essential to establish a framework that allows precise prediction of CRISPR/Cas9 editing outcome, while minimizing unwanted off-targets that promote genomic instability. Cas9 has a flexible scissile profile, which has been speculated to impact the repair outcome, however, direct evidence is lacking due to the lack of scalable methodologies that are able to probe blunt or staggered Cas9 incisions. Here we developed BreakTag, a versatile, highly parallel and scissile-aware methodology for the profiling of Cas9-induced DNA double-strand break (DSBs) at nucleotide resolution across the genome. Our results indicate that Cas9 scissile profile is not random, but instead, it is highly dependent on the nucleotide sequence of the protospacer and the presence of gRNA-DNA mismatches. Importantly, we show that Cas9-induced staggered DSBs are linked with predictable insertions, indicating that selecting gRNAs with staggered cut profile could allow prediction of repair genotypes with desirable characteristics. We are currently characterizing additional molecular determinants that generate and transform the different Cas9 cleavage profiles, focusing on the role of gRNA-DNA mismatches, of genetic variation and of downstream repair pathways. Our goal is to apply the acquired knowledge to develop an experimental framework for selection of optimal gRNAs with desirable staggered incision patterns and minimum off-target activity, for the precise and predictable deletion of any gene of interest. Our work has important implications for precise and predictable genome engineering in biomedicine and other fields of biotechnology.

SHORT TALKS 5 (ST41-45)

BIOTECHNOLOGY OF PLANTS & MICROORGANISMS

ST41

Screening a Greek collection of *Streptomyces* strains for producers of antiaging compounds

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Culture collections are a vital repository of microbial biodiversity and are also of great use to the biotechnology industry since many strains kept at collections are able to produce secondary metabolites with a wide variety of useful applications. One genus of bacteria capable of producing very large numbers of secondary metabolites, many of which are of vital importance to modern medicine and agriculture, is *Streptomyces*. Recent literature indicates that the geomorphological and climate conditions of Greece result in soil reservoirs that have a high taxonomic and functional diversity of *Streptomyces* populations, providing a rich pool of strains with potential biotechnological value. The Athens University Bacterial & Archaea Culture Collection (ATHUBA) contains a large number of *Streptomyces* isolates from Greek environments with potential industrial utility. For this reason, we screened 1000 isolates for antiaging activity. The strains were grown in liquid culture and their secondary metabolites were extracted twice using ethyl acetate followed by methanol and then tested for inhibition of elastase (which breaks down skin collagen and can cause wrinkles) and tyrosinase (which produces melanin and can cause liver spots). Our *in vitro* screen found that 1.4% of strains produced elastase inhibitors and 26.4% produced tyrosinase inhibitors and that ethyl acetate is a more efficient solvent for extraction of molecules of interest than methanol. These extracts will be further analysed and the most suitable compounds will be used in the manufacture of antiaging skin creams by an industrial partner.

ST42

Propolis: a biotechnological approach screening for antibacterial activity

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Propolis has attracted the interest of researchers in recent decades as it has a wide range of biological and pharmacological properties. It is widely used in the field of Pharmacology and Cosmetology. Propolis is a mixture of substances, sticky in nature. It is created by bees and has numerous roles inside the hive, among them the antimicrobial action. The aim of our project work was to evaluate propolis' antimicrobial activity. For this reason, standards bacterial strains were used: *Bacillus subtilis* ATCC9372, *Staphylococcus aureus* ATCC29213, *Micrococcus luteus* ATCC934 and *Escherichia coli* ATCC25922. Antimicrobial activity was determined by Disk Diffusion Assay technique. Guidelines from the Clinical and Laboratory Standards Institute were used. Only Gram positive bacteria were inhibited due to the propolis bioactive compounds, mainly due to the plant diversity grown near the hive.

We concluded that propolis has antimicrobial properties. Propolis may be a promising alternative in the treatment of some diseases according to published data that have proven many of its therapeutic activities. The pharmaceutical and cosmetic areas could benefit from this product, which could result in better disease treatment and the improvement of cosmetics with esthetic aspects.

Acknowledgments: *This work is carried out in collaboration with the Greek company Mybee which provided propolis samples*

ST43

Exploring the non-photosynthetic CO₂ assimilation in extremophilic bacteria through biomolecular network analysis and comparative genomics

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In light of the pressing need to address the “greenhouse” effect in general, and to this end, to reduce the carbon fingerprint of high CO₂-emitting industrial settings in particular, there has been a rapidly increasing interest in developing optimized engineering solutions for CO₂ capture and subsequent conversion into industrially relevant fuels and useful chemicals. The investigation of the non-photosynthetic CO₂ bioconversion has gained momentum in the field of metabolic engineering and industrial biotechnology in the recent years, as an alternative to the biomass-based microbial processes. Acetogens are the bacteria, which possess the particular ability, using catabolically the relevant Wood – Ljungdahl (W-L) pathway. *Moorella thermoacetica* is the model acetogen due to its small genome, which has been fully sequenced, while its metabolic network has been reconstructed and the relevant metabolic boundaries have been determined.

In this study, we extended the biomolecular network reconstruction of *M. thermoacetica* by determining and integrating its potentially active protein-protein interaction network based on comparative genomics analysis and systematic literature curation. Furthermore, we proceeded in investigating which bacteria possess the W-L pathway, if they can use it catabolically and under which conditions. To this end, we explored microbial biological databases and searched for the presence of W-L pathway genes in all fully sequenced bacterial genomes currently reported. The identified bacteria were prioritized with respect to a set of criteria regarding their availability, ease of use, current knowledge of their biomolecular networks and omic data available. It was validated that the W-L pathway is activated under conditions of limited or no oxygen, in the case of non-obligatory anaerobic bacteria. Cable bacteria have emerged as a promising group of bacteria, which possess the W-L pathway, and need further investigation to identify whether they could be better suitable than acetogens in the context of industrial settings with high CO₂ emissions.

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ST44

Silencing of Oleuropein β -Glucosidase Abolishes the Biosynthetic Capacity of Secoiridoids in Olives

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Specialized metabolism is an evolutionary answer that fortifies plants against a wide spectrum of (a) biotic challenges. A plethora of diversified compounds can be found in the plant kingdom and often constitute the basis of human pharmacopeia. Olive trees (*Olea europaea*) produce an unusual type of secoiridoids known as oleosides with promising pharmaceutical activities. We recently identified a β -glucosidase (OeGLU) that deglycosylates oleuropein leading to the subsequent production of the bioactive defensive aglycone form. The complete characterization of a gene should ideally be complemented with knockout analysis; however, this approach using the recalcitrant perennial woody Oleaceae species is extremely difficult. To overcome this drawback, we have introduced the Virus-Induced Gene Silencing (VIGS) approach to the olive tree. The method is based on Tobacco rattle virus (TRV). TRV is a bipartite, positive-strand RNA virus with TRV1 and TRV2 genomes. TRV2 genome is modified to carry a fragment of the target gene and post-transcriptional gene silencing is induced once TRV1 and TRV2 are delivered into a plant by Agroinoculation. VIGS offers an evolutionary rapid approach of gene silencing without requiring stable transformation. Here, we transiently silenced oleuropein β -glucosidase (OeGLU), an enzyme engaged in the biosynthetic pathway of secoiridoids in the olive trees. Nevertheless, plants contain a large set of β -glucosidases with different functionalities due to variable substrate specificity. The specificity of OeGLU towards the deglycosylation of oleuropein was confirmed with genetic analysis. Successful silencing of OeGLU was confirmed by RT-PCR in VIGS-treated plants. Reduction of OeGLU transcripts resulted in the absence of both upstream and downstream secoiridoids *in planta*, revealing a regulatory loop mechanism that bypasses the flux of precursor compounds toward the branch of secoiridoid biosynthesis. Our findings highlight that OeGLU could potentially serve as a molecular target to regulate the bioactive secoiridoids in olive oils.

ST45

Generation of ethanol tolerant *Saccharomyces cerevisiae* populations through Adaptive Laboratory Evolution

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In this project, we developed an innovative Adaptive Laboratory Evolution (ALE) strategy for the attainment of *Saccharomyces cerevisiae* populations which exhibit higher tolerance to ethanol toxicity. *S. cerevisiae* is the main microorganism used worldwide for bioethanol production, as well as for the production of alcoholic beverages or bread and pastries, through a biochemical process known as “alcoholic fermentation”. High ethanol concentrations that are produced during fermentation constitute a major stress for yeast cells, leading to decreased fermentation rate and reduced ethanol production. The applied ALE strategy involved cultivation of the yeast cells in culture medium with 20 g/L glucose or fructose as carbon source, followed by selection under high ethanol concentrations. The evolution lasted for 100 generations with fructose as carbon source and for 200 generations with glucose as carbon source. Two different parental strains, named *S. cerevisiae* CFB and *S. cerevisiae* BLR, were used and the resulting evolved populations obtained after 100 generations of evolution on glucose survived at 23% v/v ethanol for 1 h and at 25% v/v ethanol for 2 h, respectively, conditions under which the parental strains did not survive even after only 1 h of incubation. Two evolved populations originated from *S. cerevisiae* CFB and *S. cerevisiae* BLR after 200 generations of evolution on glucose, exhibited higher fermentation rates than their parental strains in synthetic broth with 200 g/l glucose, completing the fermentation 166 h and 130 h earlier, respectively. The ethanol yields of evolved populations were up to 25% improved compared to their parental strains, when grown in synthetic broths with high concentrations of glucose and/or fructose. Finally, statistically significant differences were obtained among the volatile contents of the wines (Assyrtiko and Roditis) produced by the evolved populations and the parental strains.

SHORT TALKS 6 (ST46-60)**CELL COMMUNICATION & SIGNALING****DEVELOPMENT & DIFFERENTIATION****CELL ORGANIZATION & FUNCTION****ST46****Alternative splicing responses to mitochondrial damage****Zoi Erpapazoglou¹, Malgorzata Rogalska², Panagiota Kafasla¹**¹*BSRC Alexander Fleming, Institute of Fundamental Biomedical Research, Athens, Greece*²*Centre for Genomic Regulation (CRG), The Barcelona Institute of Science and Technology, Barcelona, Spain*

Mitochondria are highly dynamic organelles with a crucial role in metabolism and signaling. Their function is constantly fine-tuned by changes in nuclear gene expression. Mitochondrial anterograde signaling allows the adaptation of mitochondrial physiology to specific cellular demands. Retrograde signaling to the nucleus is activated by mitochondrial damage, in order to repair mitochondrial function or bypass it. Mitochondrial dysfunction and signaling are critically involved in cancer development and progression, metastasis and chemo-resistance.

Mitochondrial signaling to the nucleus has been thoroughly connected to transcriptional control, but there is growing evidence that post-transcriptional regulation participates equally in mitochondrial stress responses.

In the present work, we address the short-term and long-term effects of mitochondrial dysfunction on spliceosome activity and alternative splicing outcome. We also describe specific alternative splicing changes induced by mitochondrial stress in gastric cancer cells and discuss how these responses contribute to mitochondrial quality control and can thus affect cancer development and progression.

ST47

A proteomics approach to evaluate *in vitro* the influence of brain endothelial cells on the physiology of the surrounding pericytes

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During embryonic development, cell-cell communication pathways carefully regulate the architecture of neural networks, the recruitment of endothelial cells (ECs) and the formation of the cerebral vasculature. Brain pericytes (BPs) are then recruited by ECs for angiogenesis processes, and mainly participate in the induction of specific properties carried by ECs improving the Blood-Brain Barrier (BBB) phenotype a few days after birth [1–3]. BBB is an important mechanism for protecting the brain from fluctuations in plasma composition and from circulating agents [4]. Many studies highlighted the impact of BPs on ECs during developmental phases and in neuropathological contexts but few have focused on the influence of ECs on BPs. Using an *in vitro* model the present study investigated the influence of ECs on the physiology of BPs compared to BPs cultured alone, using quantitative label free proteomics.

Human BPs [5] were cultured alone (BP-solo) or co-cultured (BP-coc) with ECs derived from human hematopoietic stem cells [6,7]. BP proteins were then processed for identification and quantification by Sequential Window Acquisition of all Theoretical Mass Spectra (SWATH) analysis (TripleTOF™ 5600+, Sciex). Interaction maps and KEGG pathways were determined from the protein lists by STRING and PANTHER databases respectively. The differential expression of proteins of interest was validated by Western blot and the physiology and metabolism of BP were evaluated. Overall, 2232 proteins were identified in BP-solo and 2491 in BP-coc, both conditions sharing 2034 proteins in common. SWATH analysis determined 51 proteins enriched in BP-solo and 90 enriched in BP-coc (fold change >2, p values <0.01).

This study has provided evidence that while ECs benefit significantly from BPs, BPs adapt their metabolism, cytoskeleton organization, intracellular vesicular transport events, and maturation under the influence of ECs. This work underlines the importance of the bidirectional communication between these two cell types for setting up and maintenance of the BBB.

¹ Sweeney, M.D.; Ayyadurai, S.; Zlokovic, B. V Pericytes of the Neurovascular Unit: Key Functions and Signaling Pathways. *Nat. Neurosci.* 2016, 19, 771–783, doi:10.1038/nn.4288.

² Menaceur, C.; Gosselet, F.; Fenart, L.; Saint-Pol, J. The Blood-Brain Barrier, an Evolving Concept Based on Technological Advances and Cell-Cell Communications. *Cells* 2021, 11, doi:10.3390/CELLS11010133.

³ Daneman, R.; Zhou, L.; Kebede, A.A.; Barres, B.A. Pericytes Are Required for Blood-Brain Barrier Integrity during Embryogenesis. *Nature* 2010, 468, 562–566, doi:10.1038/nature09513.

⁴ Abbott, N.J. Astrocyte-Endothelial Interactions and Blood-Brain Barrier Permeability. *J. Anat.* 2002, 200, 629–638.

⁵ Shimizu, F.; Sano, Y.; Maeda, T.; Abe, M.A.; Nakayama, H.; Takahashi, R.I.; Ueda, M.; Ohtsuki, S.; Terasaki, T.; Obinata, M.; et al. Peripheral Nerve Pericytes Originating from the Blood-Nerve Barrier Expresses Tight Junctional Molecules and Transporters as Barrier-Forming Cells. *J. Cell. Physiol.* 2008, 217, 388–399, doi:10.1002/JCP.21508.

⁶ Cecchelli, R.; Aday, S.; Sevin, E.; Almeida, C.; Culot, M.; Dehouck, L.; Coisne, C.; Engelhardt, B.; Dehouck, M.-P.; Ferreira, L. A Stable and Reproducible Human Blood-Brain Barrier Model Derived from Hematopoietic Stem Cells. *PLoS One* 2014, 9, e99733, doi:10.1371/journal.pone.0099733.

⁷ Deligne, C.; Hachani, J.; Duban-Deweere, S.; Meignan, S.; Meignan, S.; Meignan, S.; Leblond, P.; Carcaboso, A.M.; Sano, Y.; Shimizu, F.; et al. Development of a Human *in vitro* Blood-Brain Tumor Barrier Model of Diffuse Intrinsic Pontine Glioma to Better Understand the Chemoresistance. *Fluids Barriers CNS* 2020, 17, doi:10.1186/S12987-020-00198-0.

ST48

Endothelial RhoA mediates breast tumor-derived IL-8-induced transendothelial tumor cell migration and metastasis

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Cancer metastasis, the process through which tumor cells colonize in distant parts of the body, is currently a bottleneck for cancer treatment, and it is responsible for the majority of cancer-related deaths. Despite its recognized significance, and even though several anti-cancer therapies exist, no anti-metastatic treatment exists today. The endothelial barrier plays an active role in transendothelial tumor cell migration during metastasis, however, the endothelial regulatory elements of this step remain obscure. RhoA, a small GTPase that regulates actomyosin dynamics, plays a critical role on endothelial permeability affecting the outcome of diseases characterized by aberrant vascular leakage. Mechanistically, RhoA activation induces formation of focal adherens junctions and eventually leads to cell retraction. Translating those findings into the metastasis field, we hypothesized that blocking endothelial RhoA activation could strengthen endothelial cell-cell junctions, preventing cancer cell trans-endothelial migration, thus inhibiting the metastatic burden. Here we show that endothelial RhoA activation is a determining factor during this process: Endothelial RhoA was activated by paracrine tumor-derived mediators and its activation was proportional to the metastatic potential in a panel of triple-negative human breast cancer cell lines. A screening of inflammatory mediators highlighted IL-8, the expression of which is correlated with the metastatic potential, and its significance was confirmed via gain- and loss-of-function experiments and clinical data. In endothelial cells, IL-8 activated RhoA via p115GEF, while RhoA knockdown in vitro, endothelial-specific RhoA deficiency in vivo or pharmacological inhibition of the RhoA pathway abrogated the transendothelial migration of the breast tumor cells and the metastatic incidence of syngeneic breast tumors. These findings demonstrate the role of endothelial RhoA in tumor-derived paracrine mediated tumor cell transendothelial cell migration and highlight it as an anti-metastatic target.

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ST49

Pleiotrophin regulates translation in endothelial cells through c-Met and mTORC1 activation

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Pleiotrophin (PTN) is a highly conserved heparin-binding growth factor that regulates endothelial cell functions through receptor protein tyrosine phosphatase zeta 1 (PTPRZ1) and integrin alpha(v)beta(3) ($\alpha_v\beta_3$). mTORC1 coordinates cell growth and metabolism in response to environmental stimuli, such as nutrients and growth factors. It regulates protein synthesis, protein turnover and metabolism, and has been shown to be activated downstream of integrins, such as $\alpha_v\beta_3$ and $\alpha_5\beta_1$, as well as of the tyrosine kinase receptor c-Met. We have previously shown that in the absence of PTPRZ1, c-Met is activated in a β_3 integrin-dependent manner. In this work, we show that PTN activates c-Met and mTORC1 in endothelial cells, leading to enhanced protein synthesis, as measured by puromycin incorporation. PTN-induced phosphorylation of the mTORC1 downstream targets, such as p70S6K, and protein synthesis were abolished by rapamycin. A selective PTPRZ1 tyrosine phosphatase inhibitor also activates c-Met and induces p70S6K phosphorylation and translation, suggesting that PTN activates this pathway and enhances protein synthesis through PTPRZ1 tyrosine phosphatase inhibition. In endothelial cells that do not express PTPRZ1 or in which $\alpha_v\beta_3$ is blocked by a specific monoclonal antibody, protein synthesis and p70S6K phosphorylation are enhanced. Collectively, our data highlight a novel tyrosine kinase-dependent pathway that is activated by PTN possibly through PTPRZ1 and $\alpha_v\beta_3$ and among other functions, regulates translation in endothelial cells.

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ST50

Cell non-autonomous proteasome regulation attenuates proteotoxicity in distal tissues in *C. elegans*

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The proteasome is one of the main proteolytic complexes of the cell, responsible for at least 80% of the protein degradation including the rapid degradation of misfolded and regulatory proteins. It is part of a highly conserved network, namely the Proteostasis Network (PN), that is responsible for the cellular protein homeostasis (proteostasis). The PN controls the quality of proteins from synthesis to folding and degradation and has been shown to become deregulated during the progression of aging and age-related diseases, including proteinopathies. Given that the PN reinforcement has been proposed to be a promising approach in the design of preventive and/or therapeutic interventions against proteinopathies, it is critical to elucidate the regulation of the different PN components. Since different tissues are not equally affected under adverse conditions, tissue-communication through cell non-autonomous signaling pathways seems to be crucial for the proteome organismal integrity. Various players of the PN have been shown to be subjected to cell non-autonomous regulation through communication among tissues, with the exception of the proteasome where nothing has appeared so far. In this study, using *C. elegans* as a model, we investigated for the first time, whether the proteasome and its function may be regulated in a cell non-autonomous manner and the effects of such type of regulation upon proteotoxic stress. We reveal that proteasome activation in the neurons of the nematode can affect proteostasis in the muscle tissue. We also reveal that this communication requires the participation of a specific type of vesicles found in the synaptic transmission. Finally, we also show that this cell non-autonomous regulation is able to alleviate the proteotoxic phenotype in *C. elegans* models of Alzheimer's disease. In total, our results can provide important information for future targeted interventions.

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ST51

The association of L-Dopa Decarboxylase (DDC) with the autophagic pathway

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Catalysis by L-Dopa Decarboxylase (DDC), one of the major enzymes of catecholamine biosynthesis, converts L-3,4-dihydroxyphenylalanine (L-Dopa) to Dopamine (DA) and 5-hydroxytryptophan (5-HTP) to Serotonin. Apart from its role in neurotransmitter biosynthesis, DDC has been isolated from a variety of peripheral organs, including the liver, and is also implicated in cell proliferation, apoptosis, and host cell immunity to viruses. In neuronal cells, catecholamine signaling and DA auto-oxidation products influence autophagy. In turn, autophagy regulates the kinetics of DA release in presynaptic cells. Our study investigated the possible association between DDC and the autophagic pathway in non-neuronal cells by silencing or overexpressing DDC in human hepatic carcinoma cells. The levels of autophagy (induction and flux) were determined based on the detection of LC3B and p62 under starvation conditions and in the presence of autophagy or lysosome inhibitors. RT-qPCR was used to measure the levels of mRNA for DDC, LC3 and p62, western blotting was used to evaluate protein expression, and fluorescence imaging was used to determine localization. DDC silencing prevented autophagosome fusion with lysosomes at the late stage of autophagy flux, while DDC overexpression had the opposite effect. Furthermore, autophagy induction increased DDC expression, whereas inhibition of this pathway decreased DDC levels. These findings suggest an important role for DDC in metabolic and homeostatic processes.

ST52

Mechanisms related to PTPRZ1- dependent endothelial cell activation by pleiotrophin and VEGFA – Functional Implications for Angiogenesis

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Protein tyrosine phosphatase receptor zeta 1 (PTPRZ1) belongs to the transmembrane tyrosine phosphatases, is expressed by endothelial cells, and mediates the stimulatory effects of angiogenic growth factors, such as vascular endothelial growth factor A (VEGFA) and pleiotrophin (PTN), on endothelial cells. PTPRZ1 is also expressed in cancer cells and seems to regulate cell migration, cancer progression and metastasis in a cancer site-specific manner. It has been suggested that PTN interacts with the chondroitin sulphate chains of PTPRZ1 through its unstructured C-terminal tail, and with the protein core of the receptor through a yet unidentified domain. In the present work, we identified a novel PTPRZ1-binding domain of PTN that resides within its C-terminal thrombospondin repeat-I (TSR) domain and designed a synthetic peptide that significantly inhibited binding of both PTN and VEGFA to PTPRZ1 in endothelial cells. This peptide inhibited PTN- and VEGFA₁₆₅- induced signaling, endothelial cell migration and tube formation on matrigel in vitro, as well as angiogenesis in vivo. In addition, PTPRZ1 tyrosine phosphatase inhibition by a selective inhibitor, mimicked the effects of both PTN and VEGFA on endothelial cell activation and signaling, suggesting that PTN and VEGFA signal through inhibition of the PTPRZ1 tyrosine phosphatase activity. Data on the PTPRZ1 extracellular domain that is involved in PTN and VEGFA binding will also be discussed. Altogether, our data identify how PTN and VEGFA interact with PTPRZ1 to regulate endothelial cell activation and angiogenesis and also identify a novel peptide to be exploited for its potential anti-angiogenic effects.

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ST53

pH coordinates receptor binding and trafficking of the morphogen Dpp

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Morphological patterns are established according to the positional information encoded in the graded concentration profile of morphogens. Dpp is a secreted morphogen that controls patterning and growth of the developing *Drosophila* wing. During development, Dpp gradient scales proportionally to the size of the tissue thereby allowing adaptation of the position of the veins to the size of the wing. We have previously shown that expansion of Dpp gradient requires morphogen recycling and modulation of the binding properties of Dpp. As the tissue grows, endocytosed molecules return to the plasma membrane and unbind to contribute to the extracellular pool, which further diffuses throughout the tissue. Herein, we study the underlying mechanism controlling Dpp endocytosis and recycling. We show that Dpp trafficking is mediated by two different receptors that exhibit pH-dependent sensitivity to morphogen binding: at neutral pH of the extracellular space, Dpp binds preferentially to heparan sulfate proteoglycans. Following endocytosis, Dpp is delivered to its signalling receptor that now exhibits higher affinity for the ligand due to endosome acidification. Subsequently, pH neutralization along the recycling pathway allows dissociation of Dpp from the signalling receptor and re-secretion of the morphogen to the extracellular space. This mechanism of Dpp trafficking explains how a weak interaction at the plasma membrane, which is necessary for morphogen spreading, transforms into a strong intracellular binding for adequate downstream signalling response and subsequent release of the ligand to the extracellular milieu.

ST54

Targeting extracellular LOX and HSP70 by bispecific decoys modulate melanoma tumor microenvironment and prevents metastasis

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Cancer is a fibrotic disease and the excessive extracellular matrix (ECM) turnover drives disease progression, immune suppression and affects treatment response. Here, we propose that bispecific targeting of lysyl oxidase (LOX) and heat shock protein 70 (HSP70) affects ECM microenvironment and controls the metastatic ability of melanoma cancer cells. In this work we used human biopsies in order to validate the expression of LOX and HSP70 in melanoma tissues and we developed *in vitro* and *in vivo* melanoma models in order to evaluate the effect of our bispecific Fc-fused decoy receptor. Development of our Fc-fused bispecific inhibitors (AS1 & AS2) and their *in vivo* evaluation diminishes melanoma to lung metastasis formation. Thorough characterization of inhibitors proved their high binding affinity and inhibitory capacity against both biomarkers. In addition, bispecific decoys inhibit *in vitro* migration and invasion and *in vivo* melanoma metastasis to lung and diminished the circulated melanoma cells. Combinational treatment with immune checkpoint inhibitor dramatically increased the CD8+ T-cell-induced cytotoxicity. In the present study we revealed two new molecules, LOX and HSP70, for targeting fibrosis in malignant melanoma and this evidence led us to develop a bispecific inhibitor in order to eliminate their activity. The successful inhibition of LOX and HSP70 by the bispecific inhibitors led to rearrangement of tumor microenvironment and immune system profile, while they are preventing the melanoma to lung metastasis formation. Our findings suggest that dual-inhibition of LOX and HSP70 have the potential to be a new strategy for melanoma treatment by enhancing the efficacy of existing immunotherapies and propose the adaptation of this strategy to other cancers.

ST55

Mcdas localizes at centrioles to control centriole numbers in cycling and multiciliated cells

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Centriole numbers in cells are tightly controlled to ensure bipolar spindle assembly. Aberrations in centriole numbers lead to genomic instability and cancer¹. However, such aberrations can be part of the normal life-cycle of specific cell types. Multiciliated cells (MCC) best exemplify the deviation from a normal centriole cycle. They are post-mitotic cells which massively amplify their centrioles, bypassing the rule for once-per-cell-cycle centriole duplication. Amplified centrioles dock at the apical cell surface and generate motile cilia, beating in a coordinated fashion to ensure fluid flow across epithelia. The mechanisms controlling cells' choice between duplicating their centrioles once or hundreds of times remain poorly characterized. Recent studies highlighted Geminin and the evolutionarily-related proteins Mcdas and GemC1 as important regulators of this cell fate decision. They play key roles in cell cycle²⁻³ and centriole amplification in MCC⁴⁻⁸. Here, we characterized Mcdas as a novel protein important for maintaining correct centriole numbers in cells.

Mcdas depletion and over-expression experiments show that regulation of Mcdas expression is important for the maintenance of correct centriole numbers in cancer and normal cycling and S-phase arrested cells. Expansion microscopy was combined with mutant analysis to assess Mcdas mode of function. Mcdas affects the core centriole duplication machinery by interacting with the kinase PLK4. Post-translational modifications of Mcdas protein, including its phosphorylation by PLK4, highlight its importance in centriole number control. PLK4-specific phosphorylation sites on Mcdas protein were identified through mass-spectrometry analysis and their significance in centriole numbers was analyzed. Furthermore, we revealed a cytoplasmic co-localization of Mcdas with centriole proteins in MCC.

The above data suggest that Mcdas is important for centriole number control in cycling and post-mitotic MCC. We propose that Mcdas controls these two different centriole biogenesis pathways contributing to cell fate decisions.

¹ Gönczy P, *Nat Rev Cancer*, (2015) 15(11):639-52.

² Pefani DE et al, *JBC*, (2011) 286(26):23234-46.

³ Balestrini A et al, *NCB*, (2010) 12(5):484-91.

⁴ Stubbs JL et al, *NCB*, (2012) 14(2):140-7.

⁵ Kyrousi C et al, *Development*, (2015) 142(21):3661-74.



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- ⁶ Zhou F et al, *Curr Biol*, (2015) 25(24):3267-73.
- ⁷ Arbi M et al, *EMBOR*, (2016) 17(3): 400-13.
- ⁸ Terré B et al, *EMBOJ*, (2016) 35(9):942-60.



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ST56

Initial absolute basophil, eosinophil and monocyte counts as potential predictive markers in locally advanced rectal cancer

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Standard treatment for locally advanced rectal cancer (LARC) is neoadjuvant chemoradiotherapy (nCRT) followed by surgery. Complete clinical (cCR) or pathologic response is registered in up to 30% of patients, using standard fractionation and total dose (TD) of 45-50.4 Gy. The aim of this study was to evaluate the hematological predictors of response to nCRT. We prospectively included 75 patients with LARC between June 2020 and January 2022. All patients were treated with long-course CRT. RT was delivered using a new approach, volumetric modulated arc therapy-simultaneous integrated boost with TD of 54 Gy. Concomitant chemotherapy (5FU, Leucovorine) was given during first and fifth week of RT. Patients were assessed for tumor response in the 8th week after CRT completion with pelvic MRI scan and rigid proctoscopy. For patients with a cCR and initially distant located tumor no immediate radical surgery was suggested. The pathohistological response after surgery was assessed according to classification by Mandard. Responders were defined as patients with cCR and TRG1 and TRG2 postoperative categories. Non-responders were patients classified as TRG3-5. We analyzed initial hematological parameters. A cCR group without operative treatment included 12 patients, and the responders group comprised 35 patients (46.64%). When we compared responders and non-responders according to initial hematological parameters, it was found that higher level of initial basophil, eosinophil and monocyte counts were associated with unfavorable response ($p = 0.003, 0.01, \text{ and } 0.005$ respectively). According to the cut-off values obtained by ROC analysis (0.055, 0.155, 0.57 respectively), a statistically significant difference in the response was confirmed. Baseline basophil, eosinophil and monocyte counts were found to be promising predictive factors associated with response to treatment, which can be routinely determined by automatic, low-cost and minimally invasive methods.

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ST57

Lipid peroxidation-protective plasma membrane domains in quiescence

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Quiescence, the most common in nature but poorly studied cellular state, is essential for the long-term survival of microorganisms under stress or nutrient-limiting conditions. The plasma membrane of the yeast *Saccharomyces cerevisiae* contains several distinct domains, but the physiological role of this compartmentalization remains poorly understood [1]the coordination of which requires spatial and temporal organization into specialized domains of different sizes, stability, protein/lipid composition and overall architecture. Compartmentalization of the PM has been particularly well studied in the yeast *Saccharomyces cerevisiae*, where five non-overlapping domains have been described: The Membrane Compartments containing the arginine permease Can1 (MCC. The Membrane Compartment of the Arg transporter Can1 (MCC), or eisosome, is the most studied domain. MCCs expand in number and size in stationary phase and protect nutrient transporters from endocytosis, allowing efficient growth recovery upon transient nutrient starvation [2]our understanding of the mechanisms and functions of this lateral segregation remains incomplete. Here, we show that the clustering of the yeast Can1 arginine transporter into domains is dictated by its conformation and requires sustained biogenesis of complex sphingolipids. Furthermore, this clustering confers to Can1 and other transporters protection from ubiquitin-dependent endocytosis. Under nutrient-starvation conditions, this protective role is reinforced, thereby allowing cells to preserve a fraction of their nutrient transporters from bulk endocytosis and to more efficiently resume growth when replenishing compounds are available. Our study reveals nutrient-regulated protection from endocytosis as an important role for protein partitioning into membrane domains. The eukaryotic plasma membrane is compartmentalized into domains enriched in specific lipids and proteins. However, our understanding of the molecular bases and biological roles of this partitioning remains incomplete. The best-studied domain in yeast is the membrane compartment containing the arginine permease Can1 (MCC. However, the biological role of MCCs in upon long-term nutrient starvation remains unknown. We have now shown that MCCs, via hosting Flavodoxin-like proteins (FLPs) with ubiquinone oxidoreductase activity, protect quiescent cells from lipid peroxidation. Following glucose exhaustion, MCCs expand specifically in respiratory active quiescent cells [3] and the onset of respiration is required for this expansion. Eisosome-null and FLP-null cells show defective Post-Diauxic-Shift (PDS) growth and mitochondrial activity, reduced long-term survival and are hypersensitive to the lipid peroxidation-promoting linolenic acid (LNA). Our results suggest that FLPs act analogously to the mammalian ubiquinol-generating Fsp1, which protects cells from ferroptosis, an iron-dependent form of non-apoptotic cell death. [4]. Moreover, FLPs counteract lipid peroxidation in parallel with the well-established system of glutathione peroxidases GPX1/2/3. A strain simultaneously lacking the FLPs

and the GPX1/2/3 is extremely sensitive to LNA and shows increased number of PDS cells stained with the oxidized form of C11-BODIPY^{581/591} lipid peroxidation sensor. Importantly, targeted lipidomic analysis revealed that MCC-null and FLP-null quiescent cells contain several fold increased levels of (per) oxidized monounsaturated or polyunsaturated fatty acids incorporated into phosphatidylethanolamine. Our results uncover the importance of the changes in plasma membrane compartmentalization during Quiescence and its oxidation-protective roles for the fitness and the long-term survival of quiescent yeasts.

- ¹ Athanasopoulos A, André B, Sophianopoulou V & Gournas C (2019) Fungal plasma membrane domains. *FEMS Microbiol Rev* 43, 642–673.
- ² Gournas C, Gkionis S, Carquin M, Twyffels L, Tyteca D & André B (2018) Conformation-dependent partitioning of yeast nutrient transporters into starvation-protective membrane domains. *Proc Natl Acad Sci* 115, E3145–E3154.
- ³ Laporte D, Gouleme L, Jimenez L, Khemiri I & Sagot I (2018) Mitochondria reorganization upon proliferation arrest predicts individual yeast cell fate. *Elife* 7, 1–22.
- ⁴ Doll S, Freitas FP, Shah R, Aldrovandi M, da Silva MC, Ingold I, Grocin AG, Xavier da Silva TN, Panzilius E, Scheel CH, Mourão A, Buday K, Sato M, Wanninger J, Vignane T, Mohana V, Rehberg M, Flatley A, Schepers A, Kurz A, White D, Sauer M, Sattler M, Tate EW, Schmitz W, Schulze A, O'Donnell V, Proneth B, Popowicz GM, Pratt DA, Angeli JPF & Conrad M (2019) FSP1 is a glutathione-independent ferroptosis suppressor. *Nature* 575, 693–698.

ST58

Reciprocal regulation of HIF-1 α by ERK1/2 and CK1 δ leads to its association with microtubules

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Hypoxia-inducible factor 1 (HIF-1) transcriptionally activates genes mediating the cellular response to hypoxia as well as genes required for tumor growth. Regulation of the oxygen labile subunit HIF-1 α involves post-translational modifications such as hydroxylation and phosphorylation. We have previously shown that phosphorylation of HIF-1 α by ERK1/2 at Ser641/643 stimulates HIF-1 activity by promoting HIF-1 α nuclear accumulation and its association with chromatin components¹. Inhibition of this phosphorylation triggers HIF-1 α nuclear export and its association with mortalin on the surface of mitochondria². On the other hand, CK1 δ phosphorylates HIF-1 α at Ser247 and impairs the formation of an active HIF-1 α /ARNT heterodimer³. To investigate the interplay between these two antagonistic events and gain insight into their functional significance, we constructed double HIF-1 α mutants that combine mutations that either mimic (S247D, S641E) or abolish (S247A, S641/643A) phosphorylation by the two kinases. Analysis of HIF-1 α carrying the S247D and S641/643A mutations (HIF-1 α -SD/SA) has shown that is localized outside the nucleus and is transcriptionally inactive. Mass spectrometry analysis of GFP-HIF-1 α -SD/SA immuno-precipitates revealed that, in addition to its affinity for mitochondrial proteins, GFP-HIF-1 α -SD/SA was also associated with microtubular components. By employing in-vitro binding and immunoprecipitation assays using different HIF-1 α fragments, we mapped the interaction with tubulin at the HIF-1 α N-terminal domain and confirmed that CK1 δ -mediated phosphorylation increased the affinity of HIF-1 α for tubulin. Immunofluorescence microscopy experiments have further shown that endogenous HIF-1 α or the overexpressed HIF-1 α -SD/SA from colocalized with microtubules, especially during mitosis when CK1 δ accumulates on spindle structures⁴. Taken together, our results suggest that CK1 δ stimulates the association of HIF-1 α with microtubules and the mitotic spindle, possibly as a means to ensure efficient and equal delivery of HIF-1 α the daughter nuclei during cell division.

¹ Koukoulas et al. (2021) *Molecular Oncology* 15: 3468-3489.

² Mylonis et al. (2017) *J Cell Sci* 130: 466-479.

³ Kalousi et al. (2010) *J Cell Sci* 123: 2976-2986

⁴ Behrend et al. (2000) *Eur J Cell Biol* 79: 240-51

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ST59

Direct interaction between mortalin and HIF-1 α at the mitochondria inhibits apoptosis by blocking recruitment of BAX

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Hypoxia Inducible Factor 1, a heterodimer of alpha (HIF-1 α) and beta (HIF-1 β or ARNT) subunits, is a major regulator of the transcriptional response to hypoxia. However, HIF-1 α , the oxygen-regulated subunit, also exerts non-transcriptional functions through interaction with proteins other than ARNT. We have previously shown that the subcellular localization and protein interactions of HIF-1 α are controlled by ERK-mediated phosphorylation at Ser641/643. When modified at these sites, HIF-1 α is nuclear, binds to ARNT, interacts with NPM1, and activates transcription of hypoxia-targeted genes¹. In contrast, unmodified HIF-1 α is bound by CRM1, exits the nucleus, and, via its association with mortalin, is targeted to the mitochondria to form an anti-apoptotic complex². To further characterize the latter function, recombinant fragments of HIF-1 α and mortalin were used in in-vitro binding assays and immunoprecipitation experiments to map the respective binding sites. The results have shown that their interaction is direct, functional, and mediated by the substrate binding domain (SBD) of mortalin. We could additionally show that embelin, a natural product and known inhibitor of the mortalin-p53 interaction, also disrupts the mortalin-HIF-1 α association and, furthermore, removes unmodified HIF-1 α from mitochondria. Immunofluorescence microscopy and subcellular fractionation experiments demonstrated that mitochondrial dissociation of HIF-1 α either by embelin or by overexpression of a HIF-1 α peptide harbouring the mortalin binding site, leads under stress conditions to mitochondrial localization of the pro-apoptotic protein BAX. We suggest that when ERK activity is low under hypoxia, direct binding of HIF-1 α to mortalin inhibits mitochondrial recruitment of BAX and protects cells from apoptotic cell death.

References:

¹ Koukoulas et al. (2021) *Mol. Oncol.* 15: 3468-3489.

² Mylonis I, et al. (2017) *J Cell Sci.* 130: 466-479.

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ST60

The function of EXOSC10 sumoylation and its impact on cancer cell adaptation to hypoxia

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Sumoylation is a key post-translational modification with important roles in the adaptation and survival of cells to different types of stress including hypoxia, which characterizes major diseases such as cancer, ischemia and inflammation. The master regulators of the hypoxic response (Hypoxia Inducible factors, HIFs) can be directly modified by sumoylation or their activity can be indirectly affected by alterations in the sumoylation status of proteins participating in the hypoxic response¹. We have previously shown by mass spectroscopy and immunoprecipitation experiments that hypoxia strongly decreases the sumoylation levels of Exosome subunit 10 (EXOSC10)². EXOSC10, a catalytic subunit of the RNA exosome, is involved in the processing and degradation of various RNA species. Exploring the sumoylation mechanism of EXOSC10, we have identified the sumo isopeptidases acting on EXOSC10 and shown that it is desumoylated in a HIF-independent manner. Hypoxia caused the relocation of EXOSC10 from the nucleolus to the nucleoplasm, which was, however, not triggered by its desumoylation. Using an inducible expression system of wild-type or sumo-deficient forms of EXOSC10 in cancer cells, we analyzed the processing of rRNA precursors under both normoxia and hypoxia, but we detected no effect of EXOSC10 sumoylation or hypoxia on rRNA maturation. We are currently investigating the effect of EXOSC10 sumoylation on RNA degradation and RNA exosome protein interactions, using RNA-Sequencing and immunoprecipitation analysis, and examining the role of EXOSC10 in cell adaptation and survival under low oxygen conditions. Our results are providing new insights into the crosstalk between RNA degradation and hypoxia, which may identify new control points that can be targeted by innovative molecular therapeutic interventions.

¹ Filippopoulou et al., *Cells* 2020, 9, 2359

² Chachami et al., *Mol. Cell. Proteomics* 2019, 18, 1197–1209.

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SHORT TALKS 7 (ST61-66)

SYSTEMS BIOLOGY & BIOINFORMATICS

ST61

Machine learning assisted analysis on TCR profiling data from COVID-19-convalescent and healthy individuals unveils cross-reactivity between SARS-CoV-2 and a wide spectrum of pathogens and other diseases

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During the last two years, the emergence of SARS-CoV-2 has led to millions of deaths worldwide, with a devastating socio-economic impact on a global scale. The scientific community's focus has recently shifted towards the association of the T cell immunological repertoire with COVID-19 progression and severity, by utilising T cell receptor sequencing (TCR-Seq) assays. The Multiplexed Identification of T cell Receptor Antigen (MIRA) dataset, which is a subset of the immunoACCESS© study, provides thousands of TCRs that can specifically recognize SARS-CoV-2 epitopes. Our study proposes a novel Machine Learning (ML) assisted approach for analysing TCR-Seq data from the antigens' point of view, with the ability to unveil key antigens that can accurately distinguish between MIRA COVID-19-convalescent and healthy individuals based on differences in the triggered immune response. Some SARS-CoV-2 antigens were found to exhibit equal levels of recognition by MIRA TCRs in both convalescent and healthy cohorts, leading to the assumption of putative cross-reactivity between SARS-CoV-2 and other infectious agents. This hypothesis was tested by combining MIRA with other public TCR profiling repositories that host assays and sequencing data concerning a plethora of pathogens. Our study provides evidence regarding putative cross-reactivity between SARS-CoV-2 and a wide spectrum of pathogens and diseases, with *M. tuberculosis* and Influenza virus exhibiting the highest levels of cross-reactivity. These results can potentially shift the emphasis of immunological studies towards an increased application of TCR profiling assays that have the potential to uncover key mechanisms of cell-mediated immune response against pathogens and diseases.

ST62

Evolution of factors shaping the endoplasmic reticulum

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Endomembrane system compartments are significant elements in virtually all eukaryotic cells, supporting functions including protein synthesis, post-translational modifications and protein/lipid targeting. In terms of membrane area the endoplasmic reticulum (ER) is the largest intracellular organelle, but the origins of proteins defining the organelle and the nature of lineage-specific modifications remain poorly studied. To understand the evolution of factors mediating ER morphology and function we report a comparative genomics analysis of experimentally characterised ER-associated proteins involved in maintaining ER structure. We find that reticulons, REEPs, atlastins, Ufe1p, Use1p, Dsl1p, TBC1D20, Yip3p and VAPs are highly conserved, suggesting an origin at least as early as the last eukaryotic common ancestor (LECA), although many of these proteins possess additional non-ER functions in modern eukaryotes. Secondary losses are common in individual species and in certain lineages, for example lunapark is missing from the Stramenopiles and the Alveolata. Lineage-specific innovations include protrudin, Caspr1, Arl6lp1, p180, NogoR, kinectin and CLIMP-63, which are restricted to the Opisthokonta. Hence, much of the machinery required to build and maintain the ER predates the LECA, but alternative strategies for the maintenance and elaboration of ER shape and function are present in modern eukaryotes. Moreover, experimental investigations for ER maintenance factors in diverse eukaryotes are expected to uncover novel mechanisms.

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ST63

Sex comparative heart metabolic profiling study of PPAR β/δ -agonist GW0742 effect in a desmin-null mouse model of dilated cardiomyopathy

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Dilated cardiomyopathy (DCM) is a multifactorial disease, leading to heart failure, which is among the leading causes of mortality and morbidity worldwide. DCM progression mechanisms have been studied in the context of various mouse models. In this study, we used the desmin knockout (des^(-/-)) mouse model. Desmin is a muscle-specific protein, part of the intermediate filament network. Its absence causes DCM, leading to mitochondrial dysfunction and oxidative stress in myocardial muscle cells. The agent GW0742, agonist of the peroxisome proliferator activator receptor (PPAR) β/δ , has been indicated of having a protective effect for the heart by reducing oxidative stress and alleviating inflammation in the des^(-/-) mice. Previous studies in our laboratories have also implied a sex-specific response to the GW0742 treatment, with better results in the female compared to the male animals.

In this study, we aimed at further investigating these findings through holistic metabolic profiling of the mouse heart in the desmin-null model with (GW-treated) and without the GW0742 treatment (control) compared to the wild-type (WT), in both sexes. Untargeted gas chromatography-mass spectrometry (GC-MS) metabolomics was used, the acquired metabolic profiles were analyzed with multivariate statistical analysis tools and the results were interpreted in the context of the reconstructed mouse heart metabolic model. Indeed, this study also indicated a distinction between the GW-treated and control mouse heart metabolic profiles in both sexes, with the GW-treated profile being closer to the WT. These findings further support the protective effect of the GW0742 agonist against DCM, which appears larger in the heart of the female compared to the male animals. This may be due to the fact that PPAR β/δ is a major receptor of lipid metabolism and there are reported distinctions between sexes in the mouse heart lipid metabolism, which need to be considered in the interpretation of the acquired results.

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ST64

A comparison of 3 batch effect correction methods for harmonizing gene expression data and their correlation to the tissue proteome in bladder cancer patients

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Batch effects are defined as the change in the distribution of the data caused by non-biological factors. Because they pose a problem in data integration, several algorithms that employ statistical corrections have been designed. We compiled 12 microarray bladder cancer (BLCA) tissue datasets (n=1,139 samples) and tested the performance of three famous batch effect correction methods, namely ComBat, removeBatchEffect, and naiveRandRUV. Evaluation was based on predefined criteria, including the algorithm BatchQC, diagnostic plots, preservation of the mean expression and variance trade-off, as well as a set of 12 positive control genes with known regulation among bladder cancer conditions. BatchQC reports indicated that ComBat was able to produce high sample homogeneity achieving the highest median sample-pairwise correlation ($r_{\text{median}}=0.64$), followed by removeBatchEffect ($r_{\text{median}}=0.61$) and naiveRandRUV ($r_{\text{median}}=0.53$). In contrast to the other two, ComBat was able to minimize differences in standard deviation between samples, while also maintaining the high variance of lowly expressed genes. Of the 12 positive control genes, ComBat, removeBatchEffect and naiveRandRUV correctly identified as differentially expressed 11, 9, and 8 genes respectively. We additionally tested the three harmonized datasets against a mass-spectrometry proteomics cohort of pTa, pT1, and pT2 staged BLCA, to discover which of the three correlates better with the proteome. The analysis was conducted by correlating the Log₂FoldChange values of stage comparisons between transcriptomics and proteomics. ComBat and removeBatchEffect correlated similarly with the proteome in the T1vsTa (r=0.44 and 0.45, respectively) and T2vsT1 comparisons (r=0.19 and 0.17, respectively), while naiveRandRUV exhibited lower scores (r=0.38 and 0.08 in the T1vsTa and T2vsT1, respectively). The overall drop in the coefficients of the T2vsT1 comparison suggests an increasing discordance between the transcriptome and proteome with increasing BLCA stage, likely due to the increasing tumor heterogeneity. To summarize, ComBat outperforms both removeBatchEffect and naiveRandRUV, and can be utilized for a cross-omics analysis.

ST65

Blood Pressure Regulation Pathway Enrichment Analysis and Protein-coding Gene Prioritization through Integration of GWAS and functional data in the context of the Human Protein Interactome

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Genome-wide association studies (GWAS) have identified thousands of complex disease-associated loci. To-date, only general GWAS data repositories exist, but no disease-specific meta-databases combined with variant-gene associations. These will enable the holistic GWAS data analysis for a particular disease, in the context of biomolecular networks, upgrading their information context. This study aimed at developing a GWAS meta-database for blood pressure (BP) regulation and analyzing it in the context of the human protein-protein (PPI) network. The implemented workflow involved: (a) the development of a systematically literature-curated BP GWAS meta-database, including eQTL data and variant-gene associations, (b) the prioritization of GWAS-suggested genes based on combined criteria, (c) the reconstruction of an extended BP PPI network, identifying new BP-associated proteins as neighbors of the GWAS-suggested, (d) pathway enrichment analysis of the extended set of BP-associated proteins, and (e) prioritization of the genes in the extended set based on GWAS and network analysis criteria.

The BP GWAS meta-database includes 6687 variants, with 3738 being associated with 1167 protein-coding genes. About 25% of the genes are mapped to chromosomes 1, 2, 11 and ~57% are supported by e-QTL measurements, mostly associated with artery and nerve tibial. Associated with systolic or diastolic BP or both traits are, respectively, ~60% ~40% and 20%. Two thirds of the respective BP-proteins form a connected binary PPI subnetwork. The rest, being at most second neighbours, become connected by shortest paths, through 1443 interactors. Pathway analysis of the extended BP-protein set indicated enrichment in numerous BP-associated bio-processes. Notably, most emerged as significant after the extended PPI network reconstruction, including HIF1-pathway. Based on combined GWAS and network topology criteria, 335 BP-proteins were prioritized, 211 belonging to the 1443 network-suggested. Indeed, integrated GWAS and PPI network analysis extends our knowledge about BP regulation. The implemented workflow could be accordingly used for other multifactorial diseases.

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ST66

Network Entropy in Cancer Protein Interaction Networks of Gefitinib-resistant and Sensitive NSCLC Cells

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In this study we explore network entropy and network properties, with a systems biology approach to characterize gefitinib chemoresistance in non-small cell lung cancer (NSCLC) in terms of protein-interaction network differences and identify key factors in chemoresistance mechanisms. Network entropy increase is a characteristic of tumor cells when compared to normal cells. Cancer protein-protein interaction networks (PINs) share properties with evolving networks, exhibiting higher entropy and robustness in time. NSCLC is the second most common cancer type and one of the leading death causes worldwide in 2020. In NSCLC, epidermal growth factor receptor (EGFR), a receptor tyrosine kinase, is often overexpressed or mutated and is a therapy target. However, most patients develop acquired resistance to EGFR Tyrosine Kinase Inhibitors, leading to poor clinical outcomes and, in most cases, to death. We have identified differentially expressed genes (DEGs) from sensitive and resistant NSCLC cell lines after Gefitinib administration for 24 hours. Then we constructed protein interaction networks, using experimentally validated interactions between proteins expressed by the DEGs. Network analysis reveals that network entropy increases in time after Gefitinib administration in both resistant and sensitive NSCLC cell lines. We also find that, after chemotherapy, the small-world properties of the networks diminish in time in both sensitive and resistant cells. We demonstrate that EGFR, VAV3, and δ -catenin may synergistically regulate the activity of Rho family GTPases and, therefore, regulate downstream actin cytoskeletal dynamics in cells, which could contribute to phenotypic switching in NSCLC. Lastly, our data suggest a potential interplay between EGFR/Notch/VAV/ HIF pathways that could be mediated by δ -catenin and may be associated with chemoresistance in NSCLC.

SHORT TALKS 8 (ST67-72)

REGULATION OF GENE EXPRESSION & EPIGENETICS

ST67

The role of Ets-2 and Foxp3 interplay in T helper cell development and function

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Ets-2 is a transcription factor (TF) involved in several biological functions, including cell development, differentiation, mitosis, apoptosis, and regulation of immunity. In particular, Ets-2 suppresses the expression of IL-2 and other cytokines in effector T helper (Th) cells. Foxp3 is a TF that plays an essential role in Th cell differentiation, being a critical regulator of the development and suppressive function of regulatory Th cells (Tregs). In silico analyses revealed putative Ets-2 and Foxp3 binding sites at the promoters of Foxp3 and Ets-2, respectively. In this work, we investigated the effect of Ets-2 on the regulation of Foxp3 expression and vice versa. To this end, Jurkat T cells (a Th cell line) were transfected with increasing amounts of an Ets-2 (pCDNA3-ets-2) and a Foxp3 overexpressing vector (pRV.GFP FOXP3) in the presence (P/I) or absence (CM) of the mitogens PMA and ionomycin. Gene expression of Ets-2 and Foxp3 was examined at the transcriptional level by real-time qPCR and at the protein level by Western immunoblotting. Chromatin immunoprecipitation (ChIP) analysis was performed to investigate the in vivo binding of Ets-2 to the Foxp3 promoter. Our results showed that overexpression of Ets-2 resulted in reduced Foxp3 levels under both CM and P/I conditions. Moreover, overexpression of Foxp3 downregulated the expression of Ets-2 under both CM and P/I conditions. ChIP analysis revealed that Ets-2 binds to the Foxp3 promoter under P/I conditions. In conclusion, Ets-2 downregulates Foxp3 expression in activated Th cells by directly binding to its promoter. Our results suggest a suppressive role of Ets-2 in the development and function of Tregs and provide a basis for studies to investigate whether abnormal cross-regulation between Ets-2 and Foxp3 is involved in the pathogenesis of autoimmune diseases.

References:

- Panagoulas I, Georgakopoulos T, Aggeletopoulou I, Agelopoulos M, Thanos D, Mouzaki A. Transcription Factor Ets-2 Acts as a Pre-induction Repressor of Interleukin-2 (IL-2) Transcription in Naive T Helper Lymphocytes. *J Biol Chem.* 2016; 291(52):26707-26721.
- Toumpeki C, Anastasakis D, Panagoulas I, Stamatopoulou V, Georgakopoulos T, Kallia-Raftopoulos S, Mouzaki A, Drinas D. 2018. Construction of an M1GS ribozyme for targeted and rapid mRNA cleavage; application on the Ets-2 oncogene. *Med Chem.* 2018; 14(6):604-616.
- Davoulou P, Aggeletopoulou I, Panagoulas I, Georgakopoulos T, Mouzaki A. Transcription factor Ets-2 regulates the expression of key lymphotropic factors. *Mol Biol Rep* 2020; 47(10):7871-7881.

ST68

Comparative structural analysis of T-box riboswitches from pathogens reveals structural peculiarities with role against antibiotic resistance

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T-box riboswitches are important bacterial structured noncoding RNAs that maintain nutritional homeostasis through regulating bacterial transcription or translation¹. We have characterized several T-box riboswitches from prominent human pathogens, both structurally and functionally. Although the detailed structures have described common conserved features that are essential, T-boxes display remarkable structural diversity, especially among bacterial pathogens. For instance, we showed that in staphylococci, all glyS T-boxes synchronize glycine supply during protein synthesis and cell wall formation and are characterized by a conserved and unique insertion in their antiterminator/terminator domain, termed stem Sa. Stem Sa can accommodate binding of specific antibiotics, which in turn induce robust and diverse effects on T-box-mediated transcription². Domain swap mutagenesis and probing analysis unraveled the role of stem Sa as a requirement for efficient staphylococcal glyS T-box-mediated transcription and for binding selectivity among the tRNA isoacceptors. Moreover, stem Sa represents a lineage-specific structural feature that serves as a species-selective druggable target through its ability to modulate antibiotic binding³. Thus, a broader diversity of structural features among different T-boxes reflects the variation in metabolic adaptation among pathogens. We cloned and tested the transcription readthrough activity of the glyS T-boxes from *Listeria monocytogenes*, *Streptococcus pyogenes*, *Streptococcus pneumoniae* and *Clostridium tetani*, as representatives of prominent human pathogens. In all cases, the predicted T-box secondary structures revealed species-specific idiosyncrasies, except for the *L. monocytogenes* glyQ T-box which is bacillus-like. All four T-boxes responded to glycine starvation in vivo, as reported by the fluorescence intensity of the expressed dTomato gene under the control of each T-box. Moreover, we identified a synthetic compound that inhibits *S. aureus* growth by inhibiting the transcription rates of T-box riboswitches and their regulated genes. Overall, the broader characterization of several T-boxes from a variety of pathogenic bacteria informs the development and optimization of T-box targeted antibiotics.

References:

- ¹ Li S. et al. (2019) *Nat Struct Mol Biol* 26: 1094–1105
- ² Stamatopoulou V. et al. (2017) *Nucleic Acids Res* 45: 10242-10258
- ³ Giarimoglou N. et al. (2022) *Nucleic Acids Res* 50(10): 5834-5849

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ST69**A study of non-coding RNA transcripts that regulate the reproductive ability of female *Aedes albopictus* mosquitoes****Lefkothea- Anna Katsiaman^{1*}, Vassiliki Chatzi¹, Kostas Mathiopoulos¹**¹*Department of Biochemistry and Biotechnology, University of Thessaly, Larissa*

Long non-coding RNAs (lncRNAs) have recently emerged as a topic of major interest among the global scientific community. The reason for the intense interest researchers show in these molecules (and the genes from which they derive) stems mainly from their species-specificity and the various ways in which they can influence the final phenotype. In the context of this dissertation, we are going to study the role of some such molecules in the phenotype of oviposition, egg hatching rate and mating behavior of female *Ae. albopictus* mosquitoes. This study is an evolution of a previous dissertation carried out in our laboratory which showed that silencing of a specific lncRNA gene resulted in reduction of the reproductive capacity of female insects by more than 70%. Since most lncRNA genes of *Ae. albopictus* remain unknown we believe there are corresponding genes that control the availability of female insects in mating and which if targeted, in combination with genes involved in oviposition and hatching rate, will rapidly reduce the population of this huge public health threat. Such non-coding genes will first be searched and silenced to confirm that they actually affect the final phenotype. This will be followed by CRISPR/Cas9 knock-out of those genes that lead to the most striking phenotype of reduced reproductive capacity of insects in order to prove beyond any doubt their effect on the final phenotype. With our ultimate goal being the protection of our ecosystem, the final step of our study will be the precise, guided silencing of target genes through the modern precision-guided SIT (pgSIT) technique. This technique is based on the release of genetically modified eggs and ensures that any intervention in the insect genome will not escape the wild population.

ST70

ER β guides triple-negative breast cancer cell behavior and tumor growth *in vivo*

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Breast cancer is closely related to abnormal extracellular matrix (ECM) accumulation, reinforcing cancer cell initiation and metastasis. The complex cellular interplay within the tumor microenvironment is critical for matrix reorganization and cancer cell growth. Estrogens and their receptors (ERs) have pivotal roles in breast cancer progression. ER β has been recently implicated in matrix expression, epithelial-to-mesenchymal transition (EMT), expression of microRNAs (miRNAs) and, according to our preliminary data, exosome biogenesis. ER β suppression in MDA-MB-231 breast cancer cells reduces their aggressive phenotype through the inhibition of EMT, striking changes in their functional properties and expression patterns of major ECM mediators; however, cell population of transfected MDA-MB-231 cells demonstrated a significant heterogeneity. In the present study, we evaluated the functional role of ER β suppression following clone selection in breast cancer cells transfected with shRNA against human ER β (ESR2) that resulted in 90% reduction of ER β mRNA and protein levels. We demonstrated that ER β suppression resulted in much more reduced levels of the aggressive functional properties of MDA-MB-231 cells, followed by significantly reduced tumor growth *in vivo*. Moreover, these changes were accompanied by important alterations in the protein levels and localization of major EMT biomarkers (i.e., E-cadherin and vimentin) as well as critical ECM mediators, including syndecans, metalloproteinases, cell surface receptors and MAP kinases. An important deregulation of epigenetic signatures (i.e., miR-10b and miR-200b) has been also identified, capable of reigning over breast cancer cells properties. These novel data highlight the promising role of ER β targeting in future pharmaceutical approaches for managing the aggressive breast cancer.

ST71

ELAC1 paralogue gene encodes a ribonuclease responsible for processing of Pol III ncRNAs

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Ribonuclease Z (RNase Z) is the endonuclease responsible for the removal of 3' trailer sequences from tRNA precursors. The human genome encodes two enzymes, RNase Z^L (92.2 kD) encoded by the ELAC2 gene, and the RNase Z^S (40 kD) encoded by the paralogue ELAC1 gene. Although the role of RNase Z^L has been extensively characterized in the maturation of nuclear and mitochondrial tRNAs, the generation of tRNA-derived fragments 1 (tRF-1s) and the maturation of lncRNAs such as MALAT1¹, knowledge on the biological role of ELAC1/RNase Z^S, which is found in the cytoplasm is limited^{2,3}. Interestingly, CRISPR-Cas9 generated ELAC1 knockout cells are viable, but exhibit lower proliferation rate, limited migration capacity and altered morphology. Subsequent NGS analysis showed an overall downregulation of important genes implicated in the cell cycle regulation and motility. These changes were in most cases reversed during rescue experiments as shown by whole transcriptome sequencing which also revealed the differential expression of important classes of ncRNAs such as snoRNAs, miRNAs, tRNAs and tRNA-derived fragments. To elucidate the substrate repertoire of ELAC1 we performed CLIP-seq analysis using the WT enzyme and a catalytic null mutant. The analysis unveiled interactions not only with tRNAs, but a plethora of primarily RNA polymerase III transcripts with the most abundant being vault RNAs (vtRNAs), that are implicated in a plethora of processes like autophagy, apoptosis and proliferation. Follow-up in vitro biochemical assays revealed 3' terminal processing activity of most of the identified ncRNAs in a similar manner to tRNAs. Structure modelling of the ELAC1-ncRNA ribonucleoprotein structures showed that all substrates exhibit tRNA mimicry and can interact with the RNA binding domain similarly to tRNAs. These latest findings implicate ELAC1 in the biogenesis and processing of important Pol III transcripts thus modulating their downstream roles in translation, apoptosis, signaling, stress response and more.

References:

¹ Siira SJ et al. (2018) *EMBO Rep.* pii: e46198

² Rossmanith W. (2011) *PLoS One* 6: e19152

³ Yip MCJ et al. (2020) *Cell Rep.* 30:2106-2114

ST72

Human transcription factor LRF regulates lncRNA and miRNA species' expression

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Human LRF (Leukaemia/lymphoma related), encoded from the ZBTB7A gene is a key epigenetic player in haemoglobin switching from fetal to adult stage. Apart from γ -globin genes inhibition co-directed with the master repressor BCL11A and BGLT3-lncRNA enhancement to promote open chromatin configuration, LRF shows broad binding preferences across genome, mainly at CG-rich and CpG island (CGI) containing promoters. We extended our research to genomic loci with LRF binding sites, driving the expression of lncRNAs and miRNAs to reveal potential LRF regulatory properties on non-coding RNA species' expression. Genomic loci of preference were retrieved from ChIP-seq analysis, previously conducted¹.

Human untransfected K562 (erythroleukemia) cells and transgenic K562 clones, transfected with episomes overexpressing LRF (LRF-OE), were subjected to analysis. Genomic DNA extraction and bisulfite treatment followed by pyrosequencing uncovered methylation profiles of CGIs located at 5' prime ends of genes encoding lncRNAs. Total RNA was extracted from both untransfected and LRF-OE clones and cDNAs were tested with qPCR for selected lncRNA expression levels. Among lncRNAs, 10 with either unmethylated or not flanked by CGI showed half and less than half expression in LRF-OE clones, 3 with almost 100% methylated CGI were not expressed, while other 3 with CGI methylation between 30-80% had undetectable expression differences. 3 of the lncRNA species were outliers, which do not fall into any of the above categories. Furthermore, cDNA libraries were constructed with advanced methodology and sequenced on an Illumina i-seq 100 platform to perform both qualitative and quantitative detection of mature miRNAs. NGS results uncovered differentially expressed miRNAs between untransfected and LRF-OE clones, implicated in essential cell functions. Results of this study highlight the impact of LRF in non-coding RNAs expression regulation, mainly as a repressor. LRF exerts its indirect regulation in epigenetic events through down-regulation of non-coding RNAs and/or promoting methylation alteration in CGIs.

I. Chondrou V, Shaukat AN, Psarias G, Athanasopoulou K, Iliopoulou E, Damanaki A, Stathopoulos C, Sgourou A. "LRF Promotes Indirectly Advantageous Chromatin Conformation via BGLT3-lncRNA Expression and Switch from Fetal to Adult Hemoglobin". *Int J Mol Sci.* 2022 Jun 24;23(13):7025. doi: 10.3390/ijms23137025.

SHORT TALKS 9 (ST73-77)

CHEMICAL BIOLOGY

ST73

Evaluation of increased concentration of antibodies against egg-albumin and bovine albumin in serum and cerebrospinal fluid in patients with Alzheimer's disease

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Antibodies against food-derived antigens have been related with several diseases among which neurological disorders. Cross-reaction of these antibodies with human antigens with sequence similarity may be one of the mechanisms implicated in disease development and progression. Increased intestinal and blood brain barrier permeability, present in Alzheimer's disease (A.D.) may facilitate immunologic response against food antigens and enhance their presence in cerebrospinal fluid (CSF). In the present study, the levels of antibodies against egg-albumin, Neu5Gc, bovine casein and bovine albumin (regular, denatured and glycated) in cerebrospinal fluid and serum of healthy people and patients with A.D., were measured. For the determination, 74 CSF – 40 of patients with mild A.D. and 34 of patients with severe A.D.- and 60 serums of patients with mild, moderate and severe A.D. -20 of each group- and ELISA plates coated with the appropriate antigen were used. According to the results, 46.8% of CSF of patients with severe AD were anti-egg albumin positive and 265% increase in mean concentration was observed in severe compared to mild disease patients, in contrast with results of serums which did not show significant differences. Moreover, 45% of total of patients' serums presented high values anti-denatured bovine albumin and were observed 1.8% higher values of anti-denatured and anti-glycated bovine albumin in patients with mild A.D. compared to severe, without presenting corresponding results in the CSF of the patients. According to sequence similarity search, egg-albumin presented similarity mainly with human serpins but also with the minor histocompatibility protein and angiotensin. In conclusion, anti-egg albumin antibodies were presented in the CSF of a great portion of patients with severe A.D. indicating a probable abnormality at the function of molecules with similarity with egg-albumin, related to immunologic response.

ST74

In Vitro evaluation of 3-(benzo[d]thiazol-2-yl)-2-phenylthiazolidin-4-one derivatives for inhibition of PTP1b

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Insulin resistance is the main characteristic of patients with Diabetes Mellitus type II (DMII). In addition, imbalance in glucose-induced insulin secretion may be observed because of GLP1 (Glucagon-like-peptide-1) and GIP (Glucose-dependent insulinotropic polypeptide) impairment. Protein Tyrosine Phosphatase, PTP1b, involved in insulin receptor desensitization and Dipeptidyl Peptidase 4 (DPP4), involved in GLP-1 degradation have become drug targets for the treatment of DMII with several approved drugs of the later (1,2,3). The results presented here are part of a research aiming to the production of novel dual acting PTP1b – DPP4 inhibitors. In the present study, twenty 3-(benzo[d]thiazol-2-yl)-2-phenylthiazolidin-4-one derivatives containing the main structural characteristics and showing increased probability for dual inhibitory action according to Docking analysis, were tested *in vitro* for PTP1b inhibition. The *in vitro* evaluation was performed by colorimetric enzyme inhibition assay of p-nitrophenol. To detect the mode of inhibition, two substrate concentrations were used, 2.5mM and 30mM. According to the results, six of the compounds presented better inhibitory action at lower substrate concentration, characteristic of competitive inhibitors and eleven compounds presented characteristics of uncompetitive inhibitors. The IC₅₀ value was determined for some of the compounds and was in the μM range. Differences in inhibition percentage and in the mode of action was observed according to the presence of Mg ion, for some of the compounds.

References:

- ¹ Rolee Pathak and Mary Barna Bridgeman. *Dipeptidyl Peptidase-4 (DPP-4) Inhibitors In the Management of Diabetes*, P&T, 35 (9), 509 – 513, 2010.
 - ² Akhilesh Kumar Tamrakar et al. *PTP1B inhibitors for type 2 diabetes treatment: a patent review (2011 – 2014)*, Expert Opinion on Therapeutic Patents, 24(10), 1101-1115, 2014.
- P Eleftheriou et al. *Prediction of enzyme inhibition and mode of inhibitory action based on calculation of distances between hydrogen bond donor/acceptor groups of the molecule and docking analysis: An application on the discovery of novel effective PTP1B inhibitors*, SAR QSAR Environ Res, 26(7-9):557-76, 2015.

ST75

Identification of specific antagonists for the membrane receptor of androgens, OXER1

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Prostate cancer is known as hormone-sensitive, androgen dependent tumor and the second leading cause of cancer death in men. It is clear that androgens and androgen receptor signaling are crucial for prostate cancer growth and have been exploited therapeutically. However, hormone resistant prostate cancer is an unsolved problem with limited therapeutic choices. The action of androgens is mediated mainly through intracellular androgen receptors, which belong to the nuclear family of receptors. These receptors are transcription factors that determine key cell processes. A recent study by our team identified an alternative androgen receptor on the membrane of prostate cancer cells, OXER1 (5-oxo-6E, 8Z, 11Z, 14Z-eicosatetraenoic acid receptor). Interestingly, androgens via OXER1 inhibit cancer cell growth and migration. The aim of this research was to identify new molecules that will bind to the membrane receptor of androgens, OXER1 and will have antagonistic effects such as testosterone. To achieve this, we focused on natural products which there were data that may have a pharmacological effect and a therapeutic benefit in prostate cancer. Initially we performed in silico studies starting with the modeling of the interaction of OXER1 receptor with testosterone and 5-oxo-ETE. Due to the large number of natural products studied, an algorithm was designed and developed, allowing the fast and accurate classification of the examined chemical molecules. Next, using the advanced bioinformatics tool, OXER1 specific antagonists were identified. In vitro verification of the antagonistic properties of the selected compounds was performed in different cellular activities. The identified natural compounds, through bioinformatics methods, were tested in a number of cellular activities, related to the G_{α} and $G_{\beta\gamma}$ activities of OXER1, such as cAMP, actin polymerization and their effect on calcium ion flow. In conclusion, the achievement of present work is the identification of compounds as specific antagonists of OXER1 via G_{α} and $G_{\beta\gamma}$ activities. All these support testosterone actions at the membrane level, via OXER1, and provide new tools and agents for possible novel therapeutic approaches in cancer.

Bibliography

- ¹ Panagiotopoulos, A. et al. OXER1 mediates testosterone-induced calcium responses in prostate cancer cells. *Molecular and Cellular Endocrinology* (2022) 539: 111487.
- ² Panagiotopoulos, A. et al. New Antagonists of the Membrane Androgen Receptor OXER1 from the ZINC Natural Product Database. *ACS Omega* (2021) 6: 29664-29674.
- ³ Panagiotopoulos, A. et al. A simple open source bio-informatic methodology for initial exploration of GPCR ligands' agonistic/antagonistic properties. *Pharmacology Research & Perspectives* (2020) 8: 1-12.

This work was partially supported by Greece and the European Union (European Social Fund- ESF) through the Operational Programme (Human Resources Development, Education and Lifelong Learning) in the context of the project "Strengthening Human Resources Research Potential via Doctorate Research" (MIS-5000432), implemented by the State Scholarships Foundation (IKY) to AP (PhD scholarship), a Special Fund for Research Grants (ELKE) of the University of Crete to MK and KK and by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the "First Call for H.F.R.I. Research Projects to support Faculty members and Researchers and the procurement of high-cost research equipment grant" (Project Number: 3725 to MK).

ST76

Electrochemical Antigenic Sensor for the Diagnosis of Chronic Q Fever

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Coxiella burnetii, the causative agent of Q fever in humans, is an obligatory intracellular bacterial pathogen. Q fever is a prevalent worldwide zoonosis, which has various acute and chronic clinical manifestations. Concerning the timely diagnosis of chronic Q fever a diagnostic problem exists since the currently gold standard method for the diagnosis, immunofluorescence, has several disadvantages, such as the requirement of acute and convalescent sera, the objectivity of the interpretation of the results, potential antibody cross-reactions, the need of experienced personnel, etc. In previous studies in our laboratory, we have identified and determined antigenic proteins which could be used as a substitute for the development of an accurate serological diagnostic tool against the chronic form of the disease.

In this work, we report the development of an impedimetric biosensor for the direct, quick, and easy diagnosis of chronic Q fever. The biosensor is based on the highly sensitive antigen GroEl, that can selectively recognize antibodies against *Coxiella burnetii*. The biosensor is based on the immobilization of the antigen onto a gold electrode using the EDC/NHS immobilization methodology. The detection is performed by impedance spectroscopy that monitors specific frequencies which provide the maximum sensitivity for the biosensor. Q fever antibodies that are present in the sera of patients interact selectively with the biosensor antigens, thereby altering the impedance of the biosensor surface and generating a large impedance change within a few seconds. The biosensor allows for the specific serological detection of chronic Q fever, while the developed system can also be modified for the detection of other biomarkers, such as the ones against acute Q fever.

ST77

Titanium dioxide nanoparticle-based hydroxyl and superoxide radical production for oxidative stress biological simulations

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Hydroxyl ($\cdot\text{OH}$) and superoxide ($\text{O}_2^{\cdot-}$) radicals are key parameters responsible for oxidative stress (OS) generation in all aerobic organisms. Thus, studies exploring their oxidative effects in biological systems are of great interest. However, studying the *in vivo* effects of $\cdot\text{OH}$ and $\text{O}_2^{\cdot-}$ is unfeasible with existing methods, which can be only simulated *in vitro* at highly controlled rates of $\cdot\text{OH}/\text{O}_2^{\cdot-}$ production. This criterion is not met by the existing systems (e.g., $\cdot\text{OH}$ -producing Fenton system and $\text{O}_2^{\cdot-}$ -producing xanthine oxidase system).

We introduce a TiO_2 nanoparticle-based ($\text{TiO}_2\text{-NP}$) system for $\cdot\text{OH}$ and $\text{O}_2^{\cdot-}$ generation upon photoexcitation, to be used for *in vitro* OS biological simulations. The $\text{TiO}_2\text{-NP}$ system is set to produce $\cdot\text{OH}$ and $\text{O}_2^{\cdot-}$ alone or both, covering all possible *in vivo* generation means of these radicals. The $\text{TiO}_2\text{-NP}$ system is calibrated by monitoring the production rates of $\cdot\text{OH}$ and $\text{O}_2^{\cdot-}$ with the respective specific probes terephthalic acid and hydroethidine, which also simulate the competitive nature of free radical source/target association. $\cdot\text{OH}$ and $\text{O}_2^{\cdot-}$ production rates are linear for >60 and 8 min, respectively, while $\cdot\text{OH}$ and $\text{O}_2^{\cdot-}$ production is finely controlled by varying (i) TiO_2 concentration, (ii) excitation light-source photon-emission energy, and/or (iii) light intensity.

The linear production rates of $\cdot\text{OH}/\text{O}_2^{\cdot-}$ allow the use of the $\text{TiO}_2\text{-NP}$ system for dose-response-dependend oxidative modification studies. The biological simulating potential of the $\text{TiO}_2\text{-NP}$ system, producing both $\cdot\text{OH}$ and $\text{O}_2^{\cdot-}$, is verified on indicative biological examples structurally representing most biological systems: Bovine serum albumin (BSA), a model hydrophilic protein and low-density lipoprotein (LDL), a structure resembling most biological systems (cells, organelles, proteins, lipids). The $\text{TiO}_2\text{-NP}$ system causes a linear increase of the tested oxidative modifications on both BSA and LDL-components for exposure of 20 to 40 min, strongly suggesting that they are $\cdot\text{OH}$ dose-proportional.

References:

- ¹ Skipitari M., Kalaitzopoulou E., Papadea P., Varemменou A., Gavriil V.E., Sarantopoulou E., Cefalas, A.C., Tsakas S., Rosmaraki E., Margiolaki I., Grune T., Georgiou C.D., *J. Photochem. Photobiol. A*, 435,114290 (2023) DOI: 10.1016/j.jphotochem.2022.114290

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SHORT TALKS 10 (ST78-83)

STRUCTURE AND FUNCTION OF MACROMOLECULES

ST78

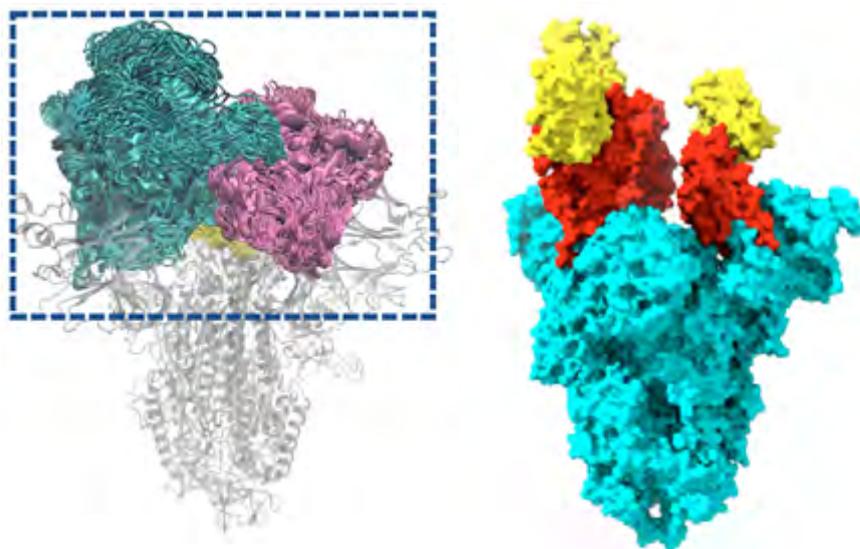
Structure-dynamics-function relationships of the SARS-CoV-2 Spike, using molecular simulations and CryoEM

Dr. Z. F. Brotzakis

Molecular simulations serve as a computational microscope into the functional motion of proteins in the atomic level. In this talk I will discuss examples of our recently developed state of the art computational-structural-biology method (MEMMI) that integrates Cryo-EM experimental data and molecular simulations to reveal the atomistic-protein-functional dynamics of spike, the main SARS-CoV-2 antigen protein. In particular, we a) reveal SARS-CoV2 virus vulnerabilities in the atomic level by identifying potentially druggable cryptic binding sites exposed during the spike protein conformational transition related to the recognition to the host cell [1] and b) find that higher affinity (function) of single domain antibody(nanobody)-spike complexes correlates with reduced conformational flexibility of the complex. With aim to to further increase nanobody affinity and therefore potency as drug, this relationship motivates to suggest and perform targeted mutations on the nanobodies that would reduce spike-nanobody conformational flexibility [2]. The mutant nanobodies-spike complexes are structurally and biophysically validated in further CryoEM and SPR experiments.

Reference:

- ¹ Brotzakis, Z. F., Lohr, T., Vendruscolo, M. Determination of intermediate state structures in the opening pathway of SARS-CoV-2 spike using cryo-electron microscopy. *Chem. Sci.* 2021, 12, 9168
- ² Mikolajek, H., M Weckener, M., Brotzakis, Z.F. et al. Correlation between the binding affinity and the conformational entropy of nanobody SARS-CoV-2 spike protein complexes". *Proc. Natl. Acad. Sci. U.S.A.* 119.31 (2022), e2205412119



ST79

Towards a structural understanding of the elevator mechanism: the case of UapA

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Structural studies of membrane proteins are exceedingly demanding using classical techniques, bottlenecked mainly by the unattainability of strongly diffracting crystals. Results of such experiments are usually high-resolution maps, albeit of singular conformations in the presence of potentially interfering detergents. Although exceedingly useful, such structures give only partial information on native, conformationally diverse proteins. Single particle analysis using electron cryo-microscopy presents an alternative pathway to structure determination. It circumvents crystallization, can facilitate a more native environment, and can also resolve conformational heterogeneity, of even a conformationally-locked mutant, from a single dataset. UapA, presents a very interesting case for structural determination, being one of the most genetically and biochemically studied eukaryotic transporters. The solved crystal structure provides a snapshot of the transport cycle, believed to function through an elevator-type mechanism. In order to pry the elusive conformational landscape, a putative Outward-Facing mutant of UapA was reconstituted in novel circular nanodiscs, and imaged by cryoEM. Preliminary results show clear 122 kDa dimers on a curved nanodisc, with single helices resolved. A more occluded conformation compared to the crystal structure, with significant movement in the “elevator” domain, further supports the current mechanism hypothesis. The same methodology can be then extended in other promising UapA mutants, but also in wild-type transporter in native lipid environments, giving a complete mechanistic insight.

Refs. Punjani, Ali, and David J Fleet. “3D variability analysis: Resolving continuous flexibility and discrete heterogeneity from single particle cryo-EM.” *JSB* vol. 213,2 (2021): 107702. doi:10.1016/j.jsb.2021.107702

Alguel, Yilmaz et al. “Structure of eukaryotic purine/H(+) symporter UapA suggests a role for homodimerization in transport activity.” *Nat.comm.* vol. 7 11336. 18 Apr. 2016, doi:10.1038/ncomms11336

Diallinas, George. “Transporter Specificity: A Tale of Loosened Elevator-Sliding.” *Trends in biochemical sciences* vol. 46,9 (2021): 708-717. doi:10.1016/j.tibs.2021.03.007

ST80

Mechanisms for allosteric inhibition of Insulin-Regulated Aminopeptidase

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Inhibition of Insulin-Regulated Aminopeptidase is being actively explored for the treatment of several human diseases and several classes of inhibitors have been reported. Here, we combine enzymological analysis with x-ray crystallography to investigate the mechanism employed by two of the most studied inhibitors of IRAP, an aryl sulfonamide, and the 2-amino-4H-benzopyran HFI-419. Although both compounds have been hypothesized to target the enzyme's active site by competitive mechanisms, we find that they instead target previously unidentified proximal allosteric sites and utilize non-competitive and uncompetitive inhibition mechanisms. X-ray crystallographic analysis demonstrated that the aryl sulfonamide stabilizes the closed, more active, conformation of the enzyme whereas HFI-419 locks the enzyme in a semi-open, but less active, conformation. HFI-419 potency is highly substrate-dependent and fails to effectively block the degradation of the physiological substrate cyclic peptide oxytocin. Our findings demonstrate alternative mechanisms for inhibiting IRAP through allosteric sites and conformational restricting and suggest that the clinical usefulness of HFI-419 may be more limited than initially considered. Such conformation-specific interactions between IRAP and small molecules can be exploited for the design of more effective second-generation inhibitors.

ST81

Macromolecular X-ray Powder Diffraction & Protein-based drug screening: High resolution structure of the pharmaceutical peptide, Octreotide

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Providing fundamental information on intra/intermolecular interactions and physicochemical properties, the 3D structural characterization of biological macromolecules is of extreme importance towards understanding their mechanism of action. Owing to recent methodological advances, XRPD is now considered as a particularly useful tool for identifying macromolecular phase transitions, quantitative analysis and determining structural modifications of samples ranging from small organics to full-length proteins ^{1,2}. To date, a series of experiments and data analyses have been carried out which establish the validity of the method³⁻⁷. In this study, an improved data collection strategy exploiting the MYTHEN II detector system together with significant beam focusing and tailored data collection options was introduced and optimized for protein samples at the Material Science (MS) beamline at Swiss Light Source (SLS) ^{7,8}. Polycrystalline precipitates of octreotide, a somatostatin analogue of pharmaceutical interest, were examined with this novel approach. XRPD experiments resulted in high angular and d-spacing (1.87 Å) resolution data (PDB code: 6vc1) ⁹, from which, electron-density maps of enhanced quality were extracted, revealing the molecule's structural properties. Since microcrystalline precipitates represent a viable alternative for administration of therapeutic macromolecules, XRPD has been acknowledged as the most applicable tool for examining a wide spectrum of physicochemical properties of such materials and perform studies ranging from phase identification to complete structural characterization.

¹ Karavassili, F.; Margiolaki, I. *PPL* 2016, 23 (3), 232.

² Spiliopoulou, M.; Valmas, A.; Triandafillidis, D.-P.; et al. *Crystals* 2020, 10 (2), 54.

³ Von Dreele, R. B. *Acta Crystallogr. D* 2001, 57 (12), 1836.

⁴ Margiolaki, I.; Wright, J. P.; Fitch, A. N.; et al. *Acta Crystallogr. D* 2005, 61 (4), 423.

⁵ Norrman, M.; Ståhl, K.; et al. *J Appl. Crystallogr.* 2006, 39 (3), 391.

⁶ Spiliopoulou, M.; Triandafillidis, D.-P.; et al. *Crystal Growth & Design* 2020, 20 (12), 8101.

⁷ Fili, S.; Valmas, A.; Spiliopoulou, M.; et al. *Acta Crystallogr. B* 2019, 75 (4), 611.

⁸ Willmott, P. R.; Meister, D.; Leake, S. et al. *J Synchrotron Rad.* 2013, 20 (5), 667.

⁹ Bergamaschi, A.; Cervellino, A.; Dinapoli, R.; et al. *J Synchrotron Rad.* 2010, 17 (5), 653.

¹⁰ Spiliopoulou, M.; Karavassili, F.; Triandafillidis, D. P.; et al. *Acta Crystallogr. A* 2021, 77 (3), 186.

ST82

Identification of a 12mer peptide ligand for human ALDH3A1 through phage library: computational prediction of protein interaction sites and *in vitro* evaluation of its ALDH3A1 inhibitory potential

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Aldehyde dehydrogenase 3A1 (ALDH3A1) is a multifunctional antioxidant enzyme, which main activity is to oxidize medium chain aldehydes to their corresponding carboxylic acids. It is also implicated in various homeostatic mechanisms, such as cell proliferation, cell cycle regulation and DNA damage response. ALDH3A1 has been characterized as a potential cancer stem cell (CSC) marker in various types of solid tumor malignancies, such as melanoma, prostate, gastric and lung cancer. Additionally, ALDH3A1 is associated with chemo/radiotherapy resistance through its ability to oxidize toxic aldehydes. In the present study, we used a random 12-mer phage peptide display library and isolated four ALDH3A1-interacting peptides, one of which (P1) appeared to bind most efficiently to ALDH3A1. Bioinformatic analysis indicated two possible P1 binding sites on the protein surface indicating biomedical potential; Site 1 which is located near the substrate binding site, implying a specific binding to ALDH3A1, and Site 2 which is overlapping with the generally conserved cofactor binding region, suggesting binding to various isoforms of ALDH enzymes' family. Moreover, we created, through BLASTp search, a list of proteins similar to P1 sequence in order to find interacting partners. Protein Kinase C Binding Protein 1 and General Transcription Factor II-I, are amongst the likely candidates due to their cellular localization and biological function. Finally, we assessed the potential ALDH3A1 inhibitory potential of the P1 peptide and demonstrated that P1 significantly inhibits recombinant human ALDH3A1's enzymatic activity against benzaldehyde. In conclusion, this study identifies a novel peptide with potential use as an ALDH3A1 inhibitor and further suggests protein candidates to be explored as possible ALDH3A1-interacting partners in future studies.

Part of the study has been conducted by the project "InTechThrace: Integrated Technologies in biomedical research: multilevel biomarker analysis in Thrace" (MIS Code 5047285), under the Operational Program "Competitiveness, Entrepreneurship & Innovation" (EPAnEK), co-funded by the European Regional Development Fund (ERDF) and national resources (Partnership Agreement 2014-2020).

ST83

The last two transmembrane segments of FurE, an APC-type fungal transporter, function as an intramolecular stabilization element via specific interactions with the core transport domain

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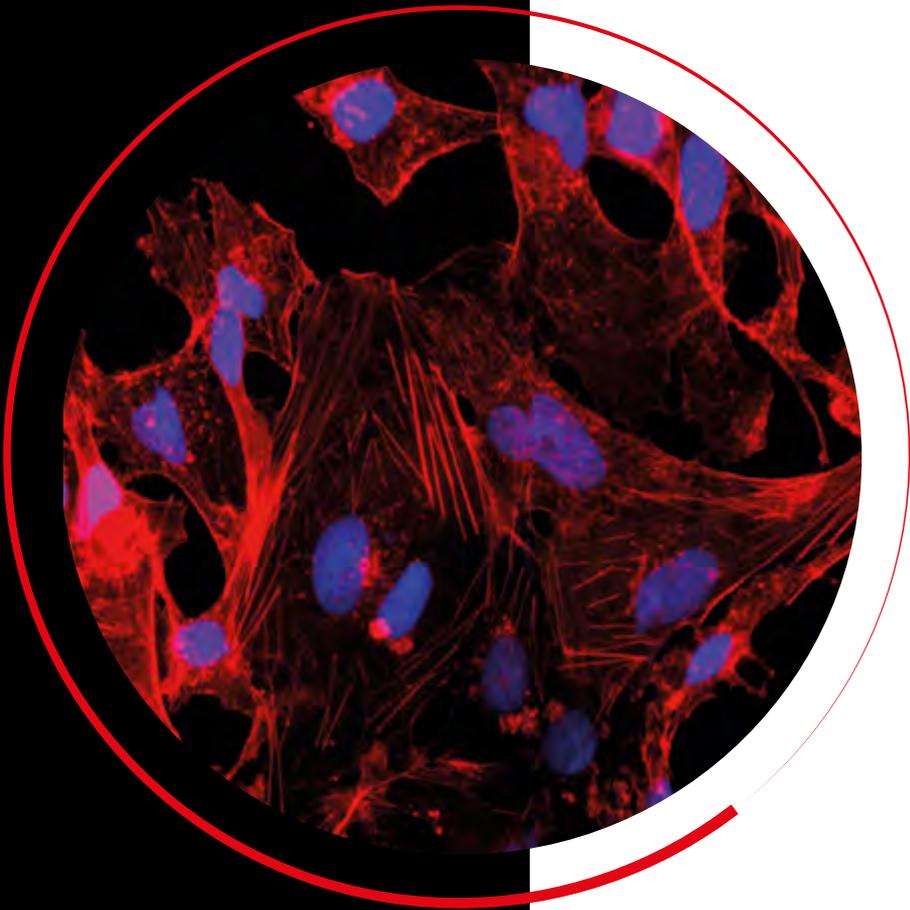
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FurE is a H⁺ symporter specific for the cellular uptake of uric acid, allantoin, uracil and nucleobase analogues of pharmacological importance (e.g. 5-fluorouracil) in the model microbial eukaryote *Aspergillus nidulans*¹. Being member of the NCS1 family, part of the APC-superfamily, FurE is characterized by 10 transmembrane segments (TMS) arranged in two inverted and intertwined repeat elements (5+5 or LeuT-fold) which form distinct 'hash' and 'bundle' domains that mediate substrate translocation through the so-called 'rocking-bundle' mechanism. Recent structural models of FurE in inward-, occluded and outward-facing conformations generated by homology modeling, have led to the identification of several residues involved in substrate/H⁺ binding, transport and specificity, and challenged some aspects of the rocking-bundle mechanism². Interestingly, however, FurE, as most APC-type transporters, contain two additional carboxyl-terminal TMSs that do not seem to participate in transport. Here we perform systematic mutagenesis of the last two TMSs of FurE (TMS11-12) and present functional analysis of relative mutants. We show that the specific identity of residues in these segments is largely dispensable for transport activity, but specific substitutions with polar residues might lead to misfolding and ER-retention. In particular, the conserved residues Y484 and W473 are shown to be irreplaceable for proper folding and ER-exit. Modeling and additional genetic evidence showed that Y484 participates in polar and hydrophobic interactions with specific residues in the 5+5-fold, essential for the structural integrity of the protein. Surprisingly, we also identify residues, proximal to extracellular and cytoplasmic loops of TMS11 and TMS12, which are crucial for substrate specificity. Our results confirm that TMS11-12 does not participate in the transport activity per se, but rather functions as a protein stabilizing domain, via specific interactions with the core transport unit of FurE.

¹ Kryptou et. al. *Mol Microbiol.* 2015 Jun;96(5):927-50.

² Zantza et. al. *bioRxiv* 2022.03.28.486045;

POSTERS





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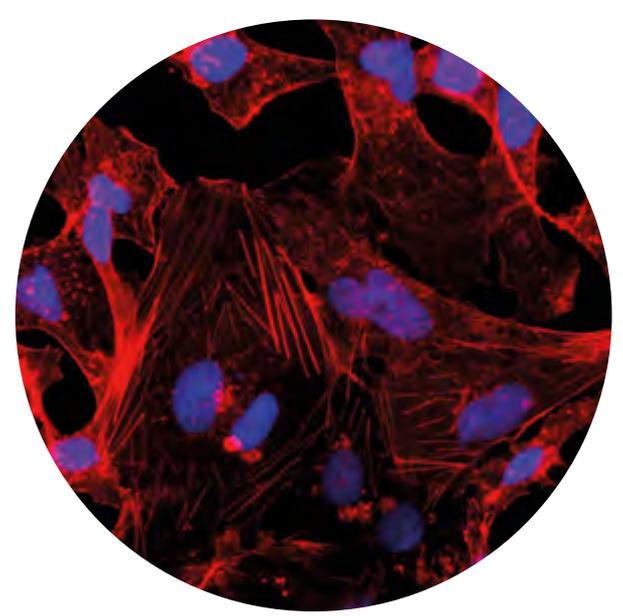
2-4 December 2022 **PATRAS**
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POSTER SESSION 1

MOLECULAR & CELLULAR BASIS OF HUMAN DISEASES

STEM CELLS, TISSUE MORPHOGENESIS & REGENERATION -

CELLULAR AGEING



P1

Amyloid Precursor Protein expression and processing is affected by L-Dopa decarboxylase inhibitors

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Diamantis C. Sideris¹, Dido Vassilacopoulou^{1*}**

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Alzheimer's disease (AD) and Parkinson's disease (PD) may share common neurodegenerative mechanisms. A hallmark pathological feature that confirms AD is the presence of extracellular plaque deposits of amyloid β peptide. In AD, it is the abnormal cleavage of the amyloid precursor protein (APP) that leads to the formation of neurotoxic amyloid plaques in the brain. PD is believed to be caused by the degeneration of Dopamine-producing cells in the substantia nigra. The neurotransmitter Dopamine is vital to motor coordination and movement. L-Dopa decarboxylase (DDC), catalyzes the decarboxylation of L-Dopa to Dopamine. PD treatment includes the administration of Carbidopa and Levodopa. Carbidopa inhibits the peripheral conversion of Levodopa to Dopamine. The goal of the current study was to investigate APP expression and proteolytic processing in cells treated with DDC inhibitors, namely, Carbidopa and NSD-1015. We found that Carbidopa increased the expression of APP in human cells of neural origin. Furthermore, the same treatment enhanced the levels of an approximate 28 kDa amyloidogenic APP proteolytic fragment. Alternatively, treatment of the same cells with the DDC inhibitor NSD-1015, significantly decreased APP expression and the levels of the 28 kDa amyloidogenic fragment. According to the data observed, L-Dopa decarboxylase inhibitors affect APP expression and amyloidogenic processing, highlighting the complexity of factors affecting APP metabolism.

P2

GATA3 protein and the NF- κ B pathway in breast cancer

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²Laboratory of Molecular Oncology, Division of Oncology, Department of Medicine, University of Patras,
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GATA3 protein has an important role in mammary gland luminal cell development and ER-positive (Estrogen Receptor) breast cancer. The NF- κ B pathway mediates a multitude of molecular and cellular functions in a large number of cells and tissues. The aim of this study is to identify a possible functional interaction between GATA3 protein and components of the NF- κ B pathway in human breast cancer. We analyzed previously published mass spectrometry data from BT474 (ER+, PR+, HER2+) breast cancer cells and performed immunoprecipitation for TRAF2 protein, an intrinsic regulator of NF- κ B, in BT474 cells. Western blot analysis for co-immunoprecipitated proteins, identified GATA3 as a TRAF2 interactor in BT474 breast cancer cells. Subsequently, we stained 10 FFPE histopathological samples from patients with ER-positive breast cancer for GATA3, TRAF2 and RelB as critical components of the NF- κ B pathway, in order to identify a possible correlation at the tissue-protein level. Scoring of tissue staining intensity and extent for GATA3, TRAF2 and RelB proteins revealed an inverse expression pattern between GATA3 nuclear localization and RelB, indicating a possible negative correlation between the two proteins. Firstly, our data cannot exclude the presence of a larger protein complex that includes other proteins besides GATA3 and TRAF3. Further, our preliminary data might point at a possible negative regulation by GATA3 on the NF- κ B pathway with important implications in breast cancer development. In any case, further studies and a larger patient cohort are needed to understand the relationship of the GATA3 protein with the NF- κ B pathway and to utilize these results in the prognosis and clinical progression of the disease.

P3

In depth proteomic characterization of the response to neoadjuvant chemoradiotherapy in locally advanced rectal cancer using data independent acquisition mass spectrometry (DIA-MS)

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Neoadjuvant chemoradiotherapy (nCRT) followed by surgery is the standard of care in patients with locally advanced rectal cancer (LARC) which increases local control and survival rates. Patients' responses to treatment differ, and it's yet unclear who will benefit the most from nCRT and is eligible for the watch and wait approach. The purpose of this study was to identify specific tissue molecular features that might influence the response to therapy and patient outcomes. Samples from 20 patients with confirmed LARC were retrospectively evaluated in this study. Patients were treated with concomitant RT/5FU+Leucovorine and divided into responders (R) and non-responders (NR) based on the tumor regression grade (TRG) (TRG1/2 vs. TRG3-5, according to the Mandard scale). Liquid chromatography/Data independent acquisition mass spectrometry (DIA-MS) was performed on digested proteins isolated from FFPE pretreatment biopsy samples, while DIA-NN, MaxQuant Perseus and Metascape were used for data processing. The analysis revealed 915 differential expressed proteins (DEPs) between responders (215 DEPs overexpressed in R/NR) and non-responders (700 DEPs overexpressed in NR/R) (Welch t-test p0.05; S0=0.1). To clarify differences in treatment response, further enrichment analysis was performed. An evident difference in signaling pathways depending on the response to therapy was observed. Ten DEPs overexpressed in responders compared to non-responders included HAS1, DERL1, CPS1, PTX4, SH2D3C, SERPINB12, CASP14, EME2, ZBTB33, GP1BB, while top 10 DEPs overexpressed in non-responder group compared to responder group included QPRT, RBP3, SPTB, MOCS2, NHLRC3, SMPDL3A, SPTA1, CLCA4, COPS7A, SYNJ2. Listed proteins have promising predictive potential and further validation of our findings in a prospective patient cohort is currently ongoing. Based on obtained results the DIA-MS approach offered unprecedented proteome coverage for FFPE samples. The detected pretreatment differentially expressed proteins and biological processes constitute interesting findings that hold the potential for improving LARC patient management.

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P4

Effect of hypertriglyceridemia on the pathogenesis of rheumatoid arthritis in a mouse model that overexpresses human apoC-III

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In chronic inflammatory diseases, such as rheumatoid arthritis (RA), morbidity and mortality are increased due to cardiovascular disease (CVD). Hypertriglyceridemia is known to disrupt the function of immune system although the implicated mechanisms are unclear. Apolipoprotein C-III (apoC-III) is an essential structural and functional component of triglyceride-rich lipoproteins (TRLs) and it is well-known for its inhibition of lipoprotein lipase. A recent study indicated that apoC-III activates the inflammasome pathway in monocytes, suggesting an active role of this protein in chronic inflammatory diseases. The aim of our research was to study the effect of hypertriglyceridemia on RA pathogenesis. As a mouse model we used the apoC-III Tg mouse strain that express at high levels human apoC-III as well as mice with identical genetic background which do not express human apoC-III (non-carriers). RA was induced by antigen-induced arthritis protocol. Serum biochemical analysis showed extremely high triglyceride and total cholesterol levels and low HDL cholesterol levels in apoC-III Tg mice compared to non-carriers [triglycerides: $1620 \pm 104,2$ vs $85,20 \pm 14,85$ mg/dL; TC: $368,8 \pm 85,23$ vs $73,24 \pm 6,307$ mg/dL; HDL-C: $8,583 \pm 2,967$ vs $49,30 \pm 2,198$ mg/dL] confirming the existence of combined dyslipidemia in apoC-III Tg mice. Density gradient ultracentrifugation of serum lipoproteins and immunoblots also confirmed the elevated human apoC-III protein levels on VLDL and HDL, and the low apoA-I levels on HDL. Furthermore, the apoC-III Tg mice developed more severe antigen-induced arthritis on knee joints which was evident at three days post injection (intra-articularly) of the antigen. Analysis of mRNA levels in peritoneal macrophages revealed noteworthy downregulation of Arg1 levels as well as increased CD36 levels in ApoC-III Tg mice. In summary, our results signify that combined dyslipidemia (high TLRs, low HDL) aggravates RA possibly via the establishment of M1-like characteristics on macrophages and increased fatty acid transportation.

P5

Regulation of the expression of the transcription factor Ets-2 in activated T cells

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Th (T helper) cells play one of the most key roles in immunity. During their induction from naive to activated they remain in a transient but very important state (Th0) before they engage to a Th effector cell fate (Th1, Th2, Th9, Tregs and more). In this transient state they produce their primary cytokine, IL-2 that is crucial for their proliferation and maturation. IL-2 production is regulated by the Ets-2 transcription inhibitor during pre-induction. Ets-2 right after activation decreases and allows IL-2 to be synthesized. Meanwhile, IL-2, due to her receptor on T cells, has the ability to induce signaling cascades and work her own regulatory plan. One of these signaling paths downstream to IL-2 includes the JAK/STAT molecules. In the end of this path, the STAT3 molecule after phosphorylation can be activated and work as a transcriptional factor. Then it can regulate gene transcription related to T cell function and IL-2 production.

In our work we used Jurkat cells as a CD4+CD25- cell model in order to study the effect of IL-2 on Ets-2 expression with the induction or blockage of IL-2 signaling in their environment. Also we tried to identify if the STAT3 downstream molecule can participate in this effect. Our observations led us to the conclusion that IL-2 signaling reduces the transcription of the pre-activation inhibitor Ets-2 and the downstream STAT3 molecule could be involved in this regulation.

References:

¹ Panagoulas, I., Georgakopoulos, T., Aggeletopoulou, I., Agelopoulos, M., Thanos, D., & Mouzaki, A. (2016). Transcription factor ets-2 acts as a Preinduction repressor of interleukin-2 (IL-2) transcription in naive T helper lymphocytes. *Journal of Biological Chemistry*, 291(52), 26707-26721. <https://doi.org/10.1074/jbc.m116.762179>

² Egwuagu, C. E. (2009). STAT3 in CD4+ T helper cell differentiation and inflammatory diseases. *Cytokine*, 47(3), 149-156. <https://doi.org/10.1016/j.cyto.2009.07.003>

³ Ng, J., & Cantrell, D. (1997). undefined. *Journal of Biological Chemistry*, 272(39), 24542-24549. <https://doi.org/10.1074/jbc.272.39.24542>

Jones, D. M., Read, K. A., & Oestreich, K. J. (2020). Dynamic roles for IL-2–stat5 signaling in effector and regulatory CD4+ T cell populations. *The Journal of Immunology*, 205(7), 1721-1730. <https://doi.org/10.4049/jimmunol.2000612>

P6

Study of Neutrophil Extracellular Traps (NETs) in *Helicobacter pylori* infection

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Helicobacter pylori (*Hp*) is a pathogen colonizing the human gastric mucosa through lifetime, increasing the risk of gastric cancer development. Although neutrophil infiltration in the lamina propria is the hallmark of *Hp* infection, it fails to clear the infecting bacteria. Considering the capacity of neutrophils to release extracellular chromatin structures (NETs) that combat infection and contribute to tissue remodeling, we investigated the presence of NETs in gastric biopsies of patients diagnosed with *Hp* chronic gastritis and respective age-matched uninfected controls by immunofluorescence confocal microscopy. NETs were identified by co-localization of myeloperoxidase (MPO) and citrullinated histone H3. Additional staining for MMP-9, MMP-3, CD47 and SIRPα was also performed. *Hp* positive biopsies demonstrated prominent presence of NETs compared to uninfected controls. Moreover, NET structures were found positive for MMP-9, while CD47 expression was found on some NETs from infected biopsies, but not SIRPα. These findings support the formation of NETs in *Hp* infection and highlight their contribution in tissue remodeling through MMP-9 matrix metalloprotease activity, while remaining in the tissue due to "do not eat me" signals. Moreover, we studied the *in vitro* interactions of freshly isolated human peripheral blood neutrophils with *Hp* strains, in terms of phagocytosis and NET formation, visualized by confocal microscopy, while ROS production was detected by flow cytometry. We documented the capacity of neutrophils to phagocytose all *Hp* strains, irrespective of CagA phosphorylation status and produce ROS, albeit at significantly lower levels to those observed when phagocytosing *E. coli*. Interestingly, *Hp* induced NETs which appeared to be degraded and demonstrated only faint presence of NET-related proteins compared to controls, suggesting possible immune-evasion of *Hp* to host neutrophilic response.

P7**NMDA - dependent cortical plasticity in neuropathic pain****Amalia Natsi¹, Thomas Mellios¹, Charalampos Labrakakis^{1,2*}**¹Department of Biological Applications & Technology, University of Ioannina, Ioannina, Greece²Institute of Biosciences, University Research Center of Ioannina (URCI), Ioannina, Greece

Pain is an aversive sensory and emotional experience that requires the combined activity of several diverse brain areas. Whilst, the sensation of pain is rather unpleasant, it is aiming to protect the organisms from dangerous and harmful stimuli. Despite the adaptive value of pain sensation, in pathological conditions such as chronic and neuropathic pain, it becomes maladaptive. Recent data suggests that chronic pain is the result of molecular changes that affect plasticity in central nervous system circuits. These changes are believed to interrupt normal connectivity and function between brain areas and lead to persisting pain.

In this study we used the spared nerve injury (SNI) neuropathic pain model and compared it with Sham operated mice, in order to investigate the modifications in cortical plasticity. We found that the NMDA dependent synchronous activity in posterior insular cortex was altered in SNI mice, indicating aberrant plastic changes.

Indeed, cortical long term potentiation deviated in SNI animals. These data imply that the development of chronic pain activates biochemical and molecular pathways that modify cellular and neural circuits in cortical areas in neuropathic pain. Further investigations might provide promising information about cellular and molecular underpinnings of pathological pain and could form the basis of improved treatment options.

P8

Establishment and characterization of chemoresistant breast cancer cell lines as *in vitro* models of drug resistance

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Triple-negative breast cancer (TNBC) is the most aggressive subtype of breast cancer; it is characterized by the lack of estrogen, progesterone and human epidermal growth factor receptors and thus, there are no targeted therapies available for this disease. Chemotherapy is the standard treatment for TNBC, but despite the initial positive response, many patients will develop drug resistance, relapse and eventually die of the disease. In the present study, we have developed and characterized two paclitaxel-resistant TNBC cell lines to be used as *in vitro* models for the identification of mechanisms that contribute to drug resistance. Paclitaxel-resistant SUM-159 and BT-549 cells were generated by exposure to escalating doses of the drug. Cytotoxicity assays were used to determine the IC₅₀ values of the resistant cell lines, which were 100- and 7.5-fold higher than those of the parental SUM-159 and BT-549 cells, respectively. The phenotype of the paclitaxel-resistant cells was more mesenchymal compared to the parental ones. Cell growth assays showed that the paclitaxel-resistant cells had a significantly slower proliferation rate compared to the parental ones. Both paclitaxel-resistant cell lines showed cross-resistance to doxorubicin. Immunostaining against β -tubulin confirmed that the resistant cells had developed mechanisms that allowed them to avoid paclitaxel damage of the microtubules. FACS analysis of the resistant cells revealed an increased efflux of doxorubicin, possibly, mediated by an increased expression of multidrug-resistance efflux pumps. RNA-sequencing revealed an enrichment in chemoresistance related pathways, such as cholesterol metabolism, drug efflux and microtubule cytoskeleton. Furthermore, BT-549 paclitaxel-resistant cells showed a high increase in breast cancer stem cells. Taken together, the above results, validate these new *in vitro* models, as useful systems to study paclitaxel resistance mechanisms in TNBC.

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P9

Pharmacological modulation of the Circadian Clock affects oncogenic characteristics of pancreatic cancer cell lines

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The circadian clock is a highly conserved autonomous mechanism of the organisms which confers 24-hour oscillations on nearly all physiological processes and has evolved as an adaptation to the environmental day/night cycles due to the Earth's rotation. At the cellular level, the circadian clock consists of a network of transcription-translation feedback loops (TTFLs) generated by a set of genes and their protein products. These clock proteins are responsible for the circadian regulation of various cellular processes including the cell cycle, apoptosis, DNA repair and metabolism, hence affecting cell fate and differentiation. Environmental and/or genetic disruption of the circadian clock is a potential cancer risk in humans and has been recently associated in genetic animal models with cancer development and progression. Moreover, clock gene or protein expression is dramatically attenuated particularly in higher stage or more aggressive tumors.

Pancreatic cancer or Pancreatic Ductal Adenocarcinoma (PDAC) is a highly aggressive human malignancy with the lowest five-year survival rate that stems from the lack of early diagnosis and ineffective treatment. RNA-sequencing and immunohistochemistry data from cancer tissue samples of PDAC patients reveal low expression of core circadian genes and proteins compared to healthy adjacent tissues, pointing to an association of the deregulation of the circadian clock with cancer progression and prognosis.

Recent drug discovery screens have identified synthetic compounds that bind to and modulate core clock proteins, providing a novel approach to cancer therapeutics. Several of these compounds that have already been tested by others, prove to be effective in inhibiting autophagy and inducing apoptosis in various cancer cell lines.

In our study we explore the effects of a recently discovered synthetic stabilizer of a clock protein on the oncogenic properties of four different PDAC cell lines. Moreover, we tested the combinatorial effects of the compound with chemotherapeutic drugs. Results concerning cell viability, cell cycle and apoptosis will be presented.

P10

Association study of IL8 and IL17A gene polymorphisms (SNPs) with the Age-related macular degeneration (AMD) in a sample population of Southwestern Greece

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Age-related macular degeneration (AMD) is a complex, progressive neurodegenerative disease and constitutes the leading cause of central vision loss in people over the age of 60, especially in the western world. AMD is divided into two main forms, dry and wet. AMD's etiology is based on the constant interaction of genetic, demographic and environmental factors. The main demographic factors are age, smoking, hypertension and race.

Concerning genetic factors, studies to date have highlighted the association of SNPs in numerous genes with disease onset and progression, most notably rs1061170 which is located in the complement factor H gene (CFH) gene. Although the pathology of the disease is not fully elucidated, it seems that inflammation plays a key role. The genes involved in inflammatory responses are a research target for the identification of AMD-related SNPs.

Interleukin genes have already been studied in different populations. In the present study, the possible correlation of SNPs rs2227306 and rs2275913 in the genes of Interleukins IL8 and IL17A, respectively, with AMD in a population sample of southwestern Greece was studied. More specifically, 92 healthy and 83 patients participated in the present study. All participants were genotyped for rs2227306 and rs2275913 SNPs by using RFLP-PCR assay.

According to the results of the present study, there was a difference in the frequencies of the rs2227306 genotypes among cases and controls. Also, there was a difference in the incidence of the two forms of AMD between the genotypes in both cases of SNPs. However, the above correlations had no statistical significance. In addition, no significant correlation was found between AMD and the simultaneous presence of the two polymorphisms. In terms of demographic factors, the one that seems to occur most frequently in patients, but without a significant correlation, is smoking and then family history.

P11

The role of fibroblasts in initiation and maintenance of inflammation in the context of insulin resistance in the model of *ligamentum flavum* hypertrophy

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Fibroblasts are active players of the innate immune system, in addition to being structural components. When activated, they secrete chemokines, cytokines and TGF β , recruiting and activating macrophages, and also produce collagen contributing to tissue homeostasis. They modify the magnitude, type, and duration of the inflammatory response and play a critical role in the switch of acute to persistent inflammation. Fibroblast accumulation and prolongation of macrophage and fibroblast activation is characteristic of fibrosis and maladaptive repair. Macrophages obtain different activation phenotypes and responsiveness to activation signals can be shaped by their microenvironment or imprinted by prior pathogenic stimuli, a condition known as 'trained immunity'. In the same context, insulin signaling modulates responsiveness of innate immune cells to inflammatory stimuli, providing a form of innate immune training. Given the established importance of fibroblasts in the initiation and maintenance of inflammation, we analyzed molecular, metabolic and epigenetic events that regulate fibroblast responsiveness to inflammatory signals and how these changes contribute to pathogenesis of connective tissue inflammation. To study the contribution of fibroblasts in inflammation we used the model of *ligamentum flavum* hypertrophy, which occurs in obese and insulin resistant patients. The results showed that fibroblasts from hypertrophic *ligamentum flavum* exhibited stronger responses to pro-inflammatory stimuli compared to fibroblasts from non-hypertrophic *ligamentum flavum*. To evaluate the contribution of insulin resistance in the phenotype we utilized the model of High Fat Diet-induced obesity and insulin resistance and fibroblasts from Akt2^{-/-} mice that are insulin resistant in the absence of obesity, to determine the role of insulin signaling in fibroblast responsiveness or 'training'. The results highlight the importance of fibroblasts in the initiation and maintenance of inflammation by acquiring a 'trained' phenotype.

P12

Role of STAT target gene networks in leukemic transformation

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Myelodysplastic Syndrome (MDS) refers to a heterogeneous group of clonal hematologic disorders characterised by hematopoietic dysfunctions and impaired differentiation. MDS is commonly referred to as a pre-leukemic stage due to increased risk of progression to acute myeloid leukemia (AML). AML is an aggressive hematologic malignancy characterised by the accumulation of immature myeloblasts in the bone marrow and the peripheral blood. Its incidence increases with age. Signal transducers and activators of transcription 3 and 5 (STAT3 and STAT5) are important regulators of several cellular processes including cell proliferation and survival. Abnormal STAT3 and STAT5 signaling has been implicated in various solid and hematologic malignancies, including MDS and AML, which renders them appealing targets for the development of novel therapeutic strategies. To elucidate the role of STAT3 and STAT5 in MDS to AML transformation, this project aims to identify changes in STAT3 and STAT5 target gene networks. To this end STAT3, STAT5A or STAT5B knock-downs have been generated in MDS and AML cell lines using shRNAs through lenti-viral delivery and mRNA-sequencing has been performed, which revealed a distinct role of the three factors in each state. It is expected that the identified target gene networks will define STAT3 and STAT5 role in leukemic transformation and will provide new targets for the therapeutic management of leukemia.

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P13

Indirubin derivatives decrease STAT3 and STAT5 phosphorylation in Acute Myeloid Leukemia cells

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Signal transducers and activators of transcription (STATs) are involved in many physiological cell functions, including hematopoiesis, differentiation, proliferation, apoptosis and survival. STATs contribute to signal transduction and regulate transcription of target genes. Their abnormal activation is linked to solid and hematologic malignancies. For this reason, the study of indirect or direct inhibition of STATs is of great interest. Indirubins are characterized by anti-cancer and anti-metastatic properties and they inhibit various kinases. They have been used to treat chronic myeloid leukemia. Here we tested indirubin derivatives for their effects on STAT3 and STAT5 action in the acute myeloid leukemia (AML) cell line, Kasumi-1. The derivatives influence viability of leukemic cells in a time and dose dependent manner and decrease STAT3 and STAT5 phosphorylation. Further studies to test the potential therapeutic use of indirubin derivatives on hematologic malignancies are ongoing.

P14

Mutations and STAT5 target genes expression: Correlation with cardiac complications in myeloproliferative neoplasms

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Myeloproliferative neoplasms (MPNs) constitute a heterogeneous group of hematological diseases. The management of patients with MPN remains largely dependent on the patient's thrombotic risk. Whether the presence of various mutations (including *JAK2*, *CARL* and *DNMT3A*) modify the likelihood of cardiac complications is not clearly understood. Abnormal signaling of Signal Transducers and Activators of Transcription (STATs) has been implicated in the pathobiology of MPNs. *JAK2* kinase activates STATs signaling and its mutation is observed in patients with cardiovascular complications. With the intention to find new molecular markers for categorization of the patients into high or low risk groups for cardiac complications in this project mutations in *JAK2*, *CARL* and *DNMT3A* and expression levels of specific STAT5 target genes have been defined. The data have been correlated with the cardiac complications observed in the patients. Our results so far, show that *JAK2* mutation is correlated with cardiac complications. This project will contribute to the categorization of patients into high or low risk groups and will generate new directions for personalized diagnostic approaches in these diseases.

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P15

The role of oxidative stress in the high osmolality-induced upregulation of calcium-activated chloride channel regulator 2 in nucleus pulposus intervertebral disc cells

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Intervertebral discs lie between the adjacent vertebral bodies of the spinal column and consist of the peripheral annulus fibrosus and the central nucleus pulposus. They are characterized by an unusual physicochemical environment, being the result of a combination of stressful conditions that ultimately contribute to their degeneration. Osmotic fluctuations are routinely faced by intervertebral disc cells - especially by those of the inner nucleus pulposus - due to the composition of the extracellular matrix itself, as well as due to the prevailing mechanical forces, with extracellular osmolality values measured *in vivo* ranging from 400 to 550 mOsm/kg H₂O.

We have shown in the past that response of nucleus pulposus cells to hyperosmotic stress involves changes in the expression of various genes, including *clca2* that encodes the calcium-activated chloride channel regulator 2 [J Cell Physiol. 2015 Dec;230(12):3037-48]. Here we show that *clca2* upregulation in nucleus pulposus cells under hyperosmotic conditions is time-dependent. Hyperosmotic stress also leads to an increase of the intracellular ROS levels, as well as of the mRNA levels of *ho-1* in nucleus pulposus cells, pinpointing to the induction of oxidative stress by high osmolality. Pretreatment of the cells with the antioxidative agent NAC abrogates high osmolality-induced upregulation of *clca2* to a great extent; furthermore, the presence of sulforaphane - an Nrf2 inducer - attenuates the high osmolality-induced *clca2* overexpression, as well, while siRNA-mediated Nrf2 knockdown further increases the already elevated by hyperosmotic stress *clca2* mRNA levels, all suggesting the implication of oxidative stress in the regulation of *clca2* expression. Nrf2 seems to be important in cellular responses towards hyperosmotic stress given that A549 cells - characterized by the constitutive activation of the particular transcription factor - are more resistant to high osmolality than intervertebral disc cells, whereas Nrf2-deficient nucleus pulposus cells become less tolerant to hyperosmotic conditions.

P16

Molecular cloning, heterologous expression, purification and antibody responses against the SARS-CoV-2 recombinant nucleocapsid (N) protein

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A new disease, named as COVID-19 (CoronaVirus Disease 2019), first appeared in the Chinese city of Wuhan in December 2019. At that time no one could imagine that this contagious disease would escalate into a global pandemic within a few months. The cause of COVID-19 is a Coronaviridae family member, the novel virus SARS-CoV-2 (Severe Acute Respiratory Syndrome CoronaVirus 2), which is a Betacoronavirus with positive sense single-stranded RNA. Numerous scientific studies have been focused on the structural proteins of SARS-CoV-2, including the second largest structural protein, the nucleocapsid protein (N protein), which is responsible for binding the viral genome and forming the viral nucleocapsid. Additionally, similar to the well-studied Spike (S) protein, the N protein is an exceptionally immunogenic component of SARS-CoV-2. The objectives of our study were the molecular cloning, heterologous expression and purification of SARS-CoV-2 nucleocapsid protein, followed by the evaluation of antibody responses, at different stages of disease, to the produced recombinant N protein, using sera from COVID-19 patients of Thrace, Greece. First, the coding region of N, derived from a Greek viral isolate, was cloned into the pBluescript plasmid followed by subcloning into a pET plasmid, in order to produce a recombinant protein with a 6xHis tag. The recombinant His-tagged N protein was expressed in E. coli cells, by induction with IPTG, and was purified, under native conditions, through affinity chromatography. Moreover, an in-house ELISA (Enzyme-Linked ImmunoSorbent Assay) was developed and was used, combined with Western blotting, to confirm the presence of antibodies against the SARS-CoV-2 recombinant nucleocapsid protein in COVID-19 patients' sera. Experiments are in progress to shed light on the immunogenic properties of the nucleocapsid protein of SARS-CoV-2, using sera from Greek Thracian COVID-19 patients.

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P17

The impact of amino acid variants of SARS-CoV-2 accessory proteins on candidate immunogenic CTL-epitopes: an *in silico* approach directed towards representative HLA alleles in the Greek population

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A novel virus, named Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), emerged towards the end of 2019 and is the etiologic agent of the respiratory disease COVID-19 (Coronavirus Disease 2019) that caused a global health crisis with great socioeconomic consequences. Thus, the scientific research has been occupied with the development of novel vaccines in order to control this unexpected pandemic. However, SARS-CoV-2, similar to other Coronaviruses, showed a highly mutated profile leading to viral escape from natural and vaccine-induced immune responses. Intriguingly, apart from the well-studied structural and functional proteins of SARS-CoV-2, a group of proteins characterized as 'accessory proteins' appear to acquire immunogenic properties and to play a crucial role in host-virus interaction. Numerous studies have focused on how structural protein mutations impair humoral immunity. On the contrary, less is known regarding T cell immune responses induced by SARS-CoV-2 proteins, especially by the accessory proteins. To this end, the objective of our study was to evaluate the impact of SARS-CoV-2 variants in accessory proteins to the CD8⁺ epitopes, predicted for the Greek population. First, we detected frequent amino acid substitutions in accessory protein sequences by multiply aligning random SARS-CoV-2 genomes of VoC (Variants of Concern) and VoI (Variants of Interest). Moreover, we identified the most representative alleles for each HLA-I (HLA-A, HLA-B and HLA-C) in the Greek population, using the Allele Frequency Net Database. The resulting data were employed to predict and compare HLA allele-specific CD8⁺ epitopes present in accessory protein variants with immunoinformatic tools, such as Tepitool, provided by the Immune Epitope Database (IEDB). Using this approach we demonstrated that many variants lead to either loss or gain (total or partial) of predicted CD8⁺ T-cell responsive peptides. Our future goal is to verify such *in silico* predictions in an *ex vivo* experimental model, in order to determine the contribution of accessory protein mutations to evading T cell immunity in infected individuals.

P18

The emergence of Oleuropein-based compounds as potent anticancer agents

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The olive tree (*Olea europaea* L.) and its products have played a significant role throughout medicinal history. Specifically, Oleuropein, a well-known glycosylated Seco-iridoid found in olive fruits and leaves, has attracted considerable scientific attention in recent years because of its biological activities and contribution to many aspects of human health. Due to the great interest in this compound, several Oleuropein and iso-Oleuropein analogues were synthesized and subsequently forwarded for bio-evaluation of their anti-cancer properties. To this end, several cancer cell models (including chemoresistant tumor cells) were treated with these compounds at various concentrations to evaluate their tumour-specific potential cytotoxic activity. In parallel, normal human skin fibroblasts and keratinocytes were used as control cells to ensure the absence of toxicity in a physiological setting. Our results showed that compounds GS29, GS32, GS33, GS34, GS36, GS37 and GS40 exerted a differential cytotoxicity against various cancer cells, including chemoresistant cell lines. The mode of action of these compounds will be further studied in preclinical tumor models.

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P19

Pharmacogenetic analysis of the mir-146a rs2910164 and mir-155 rs767649 polymorphisms and response to anti-TNF α treatment in patients with Crohn's disease and Psoriasis

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The fundamental contribution of the Tumor Necrosis α (TNF α) cytokine in the pathogenesis of Crohn's disease (CD) and Psoriasis (PsO) has established the anti-TNF α biological agents as therapeutic approaches in the clinical routine, nevertheless with diverse remission profile. Perturbed expression of regulatory elements, including microRNAs (miRs) involved in the TNF α -induced pathways, like the NF- κ B inflammatory pathway, participate in the maintenance and the response to therapy in both diseases. Therefore, our aim was to investigate putative associations between the *miR146A* rs2910164 and *miR155* rs767649 variants and response to anti-TNF α therapy in Greek patients with CD or PsO. Our multi-centre cohort consisted of 103 patients with CD and 100 patients with PsO, undergoing anti-TNF α therapy for 24 and 6 months, respectively. Disease activity and clinical remission were assessed with the Crohn's Disease Activity Index (CDAI) and Psoriasis Area Severity Index (PASI), respectively. Genotyping of both polymorphisms was conducted via the PCR-RFLP method, utilizing the de novo formation of a restriction site for the ScaI enzyme considering the *MIR146A* rs2610146, while Tsp45I was employed for the *MIR155* rs767649 variant. Statistical analysis was performed with the Stata 13.1 software. Patients' mean age was 43 (CD cohort) and 45 years (PsO cohort), with 71 (68,9%) and 68 (68%) classified as responders. The rare A allele of the *MIR155* rs767649 ($P=0.0038$) and the common G allele of the *MIR146A* rs2910164 ($P=0.0355$) were statistically associated with the response to therapy in the PsO cohort, however no association was identified in the CD cohort. The above results depict the disease-specific pharmacogenetic association of both SNPs in the response to anti-TNF α therapy in the context of PsO, while the no association found in the CD cohort may imply the differentiated expression of the NF- κ B pathway amongst CD clinical and molecular subtypes¹, where the miRs under study exhibit their action.

¹ Han, Y. M., Koh, J., Kim, J. W., Lee, C., Koh, S. J., Kim, B., Lee, K. L., Im, J. P., & Kim, J. S. (2017). NF-kappa B activation correlates with disease phenotype in Crohn's disease. *PLoS one*, 12(7), e0182071.

P20

Evaluation of miR-101 in bladder cancer prognosis and progression

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Bladder cancer (BlCa) is one of the most common malignancies in the urinary system. Due to the clinical heterogeneity of BlCa, novel markers will optimize treatment efficacy and improve the disease prognosis and management. MicroRNAs (miRNAs) are a class of small, endogenous, non-coding RNA that regulate gene expression by affecting mRNA translation and stability. Many miRNAs have been found to be deregulated during tumorigenesis and play oncogenic or suppressive roles in cancer development. In this study, we investigated miR-101 expression in BlCa and its potential prognostic significance. For this purpose, total RNA from 166 bladder tumor samples was isolated, polyadenylated at 3'-end and reversed transcribed. SYBR-Green based qPCR assays were performed for the quantification of miR-101 expression. For qPCR calculations, we applied the comparative Ct method for relative quantification; the small nucleolar RNA, C/D box 48 (SNORD48), also known as RNU48, served as endogenous control, and BJ human newborn foreskin fibroblasts, were used as calibrator. miR-101 levels were increased in muscle-invasive (T2-T4) compared to superficial tumours (TaT1), and in high compared to low-grade tumours. Our biostatistical analyses (including Kaplan-Meier survival analysis) showed that loss of miR-101 expression predicts poor disease-free survival (DFS) in TaT1 BlCa patients ($p=0.033$). Thus, TaT1 patients with tumors poorly expressing miR-101 are at significantly higher risk for relapse as compared to patients with miR-101 overexpression. In conclusion, miR-101 is heavily deregulated in bladder tumours and is a favourable molecular biomarker for TaT1 patients.

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P21

Investigating the association of ALDH1B1 with cancer stem cell-related molecules in human colorectal adenocarcinoma

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Aldehyde dehydrogenases (ALDHs) are NAD(P)⁺-dependent enzymes that catalyze the oxidation of aldehydes to their corresponding carboxylic acids. ALDH superfamily also participates in many other cellular mechanisms such as cell proliferation, differentiation and apoptosis. During the last years, ALDH have been reported as cancer stem cell (CSCs) markers. CSCs, also known as tumor initiating cells, are characterized by a set of stem-like properties, such as self-renewal, differentiation, cell survival and asymmetric cell division. CSCs are considered important for tumor heterogeneity due to their ability to differentiate into multiple cell types, while they are considered important therapeutic target as a result of their enhanced chemo- and radio-resistance as well as their association with cancer initiation, progression and metastasis. ALDH1B1 is correlated with colon tumorigenesis, pancreatic stem cells and beta cell development and involved in various CSC-related cellular signaling pathways such as PI3K/Akt, Wnt/ α -catenin and Notch. In this study, we examined the role of ALDH1B1 in CSC-signaling pathways, by evaluating the association of ALDH1B1 with CSC-associated molecules through Spearman's rank correlation coefficient using data from colorectal adenocarcinoma patient samples (531 patients with colon and/or rectal adenocarcinoma), obtained from The Cancer Genome Atlas (TCGA) database. The estimated correlation of ALDH1B1 with CSC-related molecules was further confirmed in vitro by using stably transfected cell lines (HT-29 and Caco-2) overexpressing human ALDH1B1 and performing gene expression analysis by real-time PCR. We exhibited significant up-regulation of specific genes such as *TEAD2*, *IFT74*, *DRD2*, *PTPRD*, *BRINP1*, *PDHA1*, *CLTA* in the ALDH1B1-overexpressing HT-29 and -Caco2 cells compared to mock control cells. Finally, our results support the hypothesis that ALDH1B1 may interact with CSC-related signaling pathways in colorectal adenocarcinoma and further research is warranted towards unravelling the mechanistic details of such ALDH1B1-driven interactions.

P22

The role of non-opsonic receptors in Group B Streptococcus (GBS) phagocytosis and intracellular killing in neonatal and adult macrophages

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Sepsis is the host's overwhelming and life-threatening response to infection that affects individuals of all ages. In early life, sepsis leads to substantial morbidity and long-term neurodevelopmental sequelae. Neonates and young infants are susceptible to bacterial infections, especially *Streptococcus agalactiae* (GBS). GBS is a noninvasive pathogen in immunocompetent adults but the predominant cause of sepsis and meningitis in neonates. Up to now, the innate immune mechanisms that account for the increased vulnerability of neonates to GBS remain unclear.

Receptor-mediated phagocytosis employs plasma-membrane receptors in host's phagocytes for pathogen engulfment. We performed RNA sequencing analysis of GBS-infected adult and neonatal thioglycolate-elicited peritoneal macrophages and the results showed that the phagocytosis mechanism differed significantly between neonates and adults. In particular, distinct non-opsonic receptors were upregulated after GBS infection in neonatal macrophages compared to that in adult cells. The poor antibody response induced by GBS in combination with low levels of complement components in neonates suggests that GBS internalization is mediated by non-opsonic-dependent phagocytosis. Interestingly, the scavenger receptor MARCO was elevated in neonatal macrophages, both basally and upon GBS infection, in comparison with the corresponding adult cells. The role of MARCO in GBS clearance and the mechanism initiated by non-opsonic receptors were further evaluated using MARCO-deficient macrophages.

To date, the plasma-membrane receptors that cause GBS internalization have not been characterized in neonates. The results of our study will facilitate a better understanding of the mechanisms that account for the high susceptibility of young hosts to bacterial infections, promoting the development of new diagnostic, preventive and/or therapeutic approaches to improve the poor prognosis of neonatal sepsis.

P23

Inhibition of the Akt/mTOR pathway promotes autophagy and clearance of Group B *Streptococcus* from the alveolar epithelium

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Group B *Streptococcus* (GBS) is a gram-positive bacterium that is harmless for healthy individuals but may provoke invasive disease in young infants and immunocompromised hosts. As GBS invades epithelial barriers to enter the bloodstream, strategies that enhance epithelial cell responses may hamper GBS invasion. In the present study, we investigated whether inhibition of Akt, a kinase that regulates host inflammatory responses and autophagy via suppression of mTOR, can enhance the response of non-phagocytic alveolar epithelial cells against GBS. Treatment of the alveolar epithelial cell line A549 with the Akt inhibitor MK-2206, resulted in enhanced production of reactive oxygen species and inflammatory mediators in response to GBS. Additionally, Akt inhibition via MK-2206 led to reduced phosphorylation of the mTOR targets S6 and 4E-BP1 and subsequent LC3 lipidation. Importantly, inhibition of Akt promoted GBS clearance both in alveolar epithelial cells in vitro and in lung tissue in vivo, in a murine model of GBS pneumonia. Induction of autophagy was essential for GBS clearance in MK-2206 treated cells as knockdown of ATG5, a critical component of autophagy, abrogated the effect of Akt inhibition on GBS clearance. Our findings highlight the role of Akt kinase inhibition in promoting autophagy and GBS clearance in the alveolar epithelium. Inhibition of Akt may serve as a promising measure to strengthen epithelial barriers and prevent GBS invasion in susceptible hosts.

P24

Differentiated ECM composition in 3D breast cancer cell spheroids

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Cancer cell growth and survival are orchestrated by the surrounding extracellular matrix (ECM), the three-dimensional meshwork of a wide variety of macromolecules with both structural and functional role. The molecular mechanisms of the complex interplay between the tumor cells and the tumor microenvironment play a pivotal role in cancer progression. Traditionally, 2D cell culture models are currently used as the “gold standard” for studying cellular communications *ex vivo*. However, there are essential limitations based at the fact that cell-cell interactions only occur at the edges of the cells in the 2D culture systems, 3D cell culture involves cellular stretch and interactions from all angles, as well as cell-ECM interactions. Tumor spheroids are 3D, self-assembling cell structures which are formed by cancer cells with high heterogeneity and enhanced cell-cell and cell-ECM interactions. This type of interactions in spheroids significantly mimic *in vivo* cyto-architecture in a manner which is more pathophysiologically relevant when compared to 2D monolayer cultures. Additionally, gene expression profiles of cells grown in the 3D microenvironment better mimic clinical conditions, when compared to 2D monolayer cultures. In the present study we aim at a better understanding of the role of spheroids as regulators of the expression of ECM molecules and their impact at the ECM configuration in breast cancer. Therefore, spheroids could be a useful tool in *in vitro* models due to their ability to imitate the *in vivo* microenvironment of tumors in a more realistic way compared to 2D cell cultures.

P25

Serglycin-mediated WISP-1 expression controls glioblastoma cell functions

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Glioblastoma (GBM) is the most common aggressive primary brain tumor. Serglycin is a proteoglycan that can regulate the aggressive phenotype in several tumors, including GBM. Serglycin is secreted in the extracellular matrix and activate multiple oncogenic signaling pathways. WISP1 is a secreted cysteine rich growth factor that plays a vital role in embryonic stem cells controlling their functional properties. However, WISP1 also found to contribute to the progression of various tumors. WISP1 expression is upregulated in GBM compared to nonmalignant tissues, whereas its downregulation lead to reduced cell proliferation, migration, invasion and stemness phenotype of GBM cells. WISP1 is reported to signal through integrins and activate a plethora of signaling pathways involved in tumor cell behavior. The interaction of WISP1 with $\alpha v \beta 3$ can manipulate crucial cellular functions, while its interaction with $\alpha 6 \beta 1$ can maintain the stemness capacity of glioma stem cells. Our laboratory has generated LN18 GBM cells with suppressed levels of serglycin (LN18^{shSRGN}), which are characterized by reduced proliferation and migration rates, as well as diminished ability to form tumors in vivo. The expression levels of WISP1 are downregulated in LN18^{shSRGN} cells. This is also associated with a significant reduction in mRNA and protein expression levels of various integrins. Using exogenously added recombinant WISP1, we found that LN18^{shSRGN} increase their ability to proliferate, migrate and invade as well as to form colonies. Treatment with WISP-1 also increased the expression of b-catenin and proteolytic enzymes. The increased expression of WISP-1 is also associated with reduced overall survival in GBM. It is proposed that serglycin oncogenic properties are partially mediated through the regulation of WISP1 expression in GBM cells.

P26

Signaling pathways induced by hyperglycemia in H9c2 cardiac cells

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Hyperglycemia induces a plethora of signal transduction pathways leading to diabetes, a metabolic disease with a worldwide impact. Cellular death, oxidative stress and inflammation triggered under hyperglycemic conditions contribute to diabetic complications, affecting multiple tissues and organs, including the heart [1]. With reports highlighting a possible causative correlation among cardiovascular diseases, diabetes and autophagy, the present study aimed at elucidating the relative signaling mediators involved in the response of H9c2 cells to hyperglycemic conditions. To this end, the effect of high glucose levels (25mM) on fundamental effectors of the autophagic mechanism was examined. In particular, given the established role of AMPK in initiation of autophagy, the time-dependent profile of its phosphorylation, was investigated and found to be maximal after 15-30min incubation with 25mM glucose. Interestingly, ULK-1, a known AMPK substrate, was also found to be maximally phosphorylated at 30-60min, further corroborating induction of autophagy. In agreement with the aforementioned results, a gradual degradation of p62 levels (indicative of autophagy) was also detected in our samples. Occurrence of autophagy was also substantiated by monitoring a significant increase in the levels of the LC3-II/LC3-I ratio, with LC3 lipidated and converted from its cytoplasmic LC3-I to its LC3-II form during autophagy [2]. A transient activation of members of the MAPKs, that have been linked to regulation of the autophagic flux [3], was also detected. In conclusion, diverse signaling pathways appear to finetune the ultimate response of cardiac cells to hyperglycemia. Performing an MTT assay, 24h incubation of H9c2 cells with 25mM glucose caused $\sim 40,5 \pm 1,4$ % reduction in cell viability. Evidently, further studies are required in order to fully decipher the effectors mediating the responses triggered by hyperglycemia in H9c2 cardiac cells, aiming to the identification of those with a potential to ameliorate cardiac cell survival under these stressful conditions.

¹ *Cardiovasc. Diabetol.* 2018, 17, 83

² *EMBO J.* 2000, 19, 5720

³ *J. Biol. Chem.* 2003, 278, 16667

P27

The in vitro anticancer activity of a σ_2 agonist siramesine in pancreatic cancer

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Objective: Siramesine (SRM), is an agonist of σ_2 receptors that is reported to show a promising antiproliferative and cytotoxic activity in tumor cells in vitro as well as in vivo. The aim of this study is the investigation of the in vitro activity of Siramesine both in the established human pancreatic cancer cell lines PANC-1 & BxPC3 and in an ex vivo pancreatic cancer cell population named Attached which was isolated in our laboratory directly by a patient derived xenograft.

Materials & Methods: We used the methods of SRB cytotoxicity test to determine the GI50, TGI, and LC50 of siramesine against pancreatic cancer cells, the clonogenic assay, and the wound healing assay to investigate the cytotoxic activity of siramesine, the inhibiting activity of SRM on the ability of single cells to make clones, and on the ability of the specific cells to migrate, respectively. Furthermore, through flow cytometry, we studied the effect of the compound on the cell cycle of pancreatic cancer cells to determine if the activity is cell cycle phase-specific.

Results: The data of this study, confirmed that siramesine has a strong antiproliferative and cytotoxic activity under the experimental conditions that have been tested. Moreover, siramesine was found to inhibit the ability of single cells to create colonies and to migrate in a time and dose-dependent manner. The flow cytometry data suggest that siramesine induces cell cycle arrest at the G0/1 phase of the cell cycle.

Conclusions: Siramesine, under the experimental conditions tested herein, exhibits strong anti-clonogenic and anti-migratory activity. Furthermore, we show that the activity of the compound is cell-cycle phase-specific against pancreatic cancer cells. These data are reported for the first time for siramesine. Further studies on the mechanism via which the compound exhibits these effects are ongoing.

P28

Imaging in three dimensions: an advanced pipeline for monitoring cells in multicellular tumor spheroids

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For many years, two-dimensional cell cultures have been the gold standard method for studying the behavior of cells in the context of many diseases, including cancer. Although their simplicity and easiness in handling were key advantages compared to other approaches, nowadays they are considered as limiting factors, especially in studies aiming to develop models that mimic the characteristics of an *in vivo* tumor. To this end, in the last years, researchers prefer to work with three-dimensional (3D) cell cultures, in order to achieve a greater degree of accuracy in the representation of the *in vivo* microenvironment.

Depending on their origin, different types of cancer cells may require special culture conditions, in order to form tumor-like spheres (spheroids). In order to optimize these conditions, we compared a variety of culturing protocols in our 3D cell cultures. Moreover, we designed and constructed a mechanical system, and a sample holder, that allow us to rotate the samples and acquire images from different angles in the Light Sheet Fluorescence Microscope installed at our Bioimaging Facility.

Light sheet microscopes produce large amounts of data, which usually require complicated computational tools in order to be processed. To circumvent this problem, we developed an “all-in-one” tool, via which we are can segment and track cellular nuclei in 2D and 3D time lapse videos, visualize, validate and correct results and also simplify procedures for training and applying Machine and Deep Learning models to boost analysis outcomes.

Being able to perform imaging in 3D can give great insights into the better understanding of cancer-related procedures. Thus, being able to follow a pipeline that produces accurate and reproducible results will have a positive impact on research.

P29

Acute stress responses in high anxiety: Focus on mitochondrial dynamics

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It is estimated that 300 million people are affected by an anxiety disorder. There is variability in stress perception and/or predisposition, with highly anxious individuals being more prone to stress effects. Mitochondria, the cellular metabolic hubs, are implicated in stress responses and shape anxiety phenotypes. The highly coordinated processes of mitochondrial dynamics, including mitochondrial biogenesis, fission, fusion and mitophagy, facilitate the quality control of the mitochondrial pool. However, how mitochondrial dynamics shape stress responses in high anxiety remains elusive. Here, we explore the modulatory role of brain mitochondrial dynamics in the predisposition to stress responses in a high anxiety background, by using a high anxiety-like behavior (HAB) mouse model. Adult male HAB mice were exposed to acute restraint stress and were compared to unstressed control HAB mice. Basal, pre-stress anxiety-related and depression-like behavior were evaluated by a behavioral test battery, including the elevated plus maze, the open field and the forced swim tests. Prefrontal cortex and plasma were then collected from stressed and unstressed control HAB mice for molecular analyses. Pre-stress behavioral data were also correlated with post-stress molecular readouts. Our results show that stressed HAB mice display altered gene expression of mitochondrial dynamics key players involved in mitochondrial biogenesis, fission, fusion and mitophagy compared to unstressed controls. Interestingly, gene and protein expression of mitochondrial fusion regulators is associated with the basal anxiety and depression-like pre-stress behavior in HAB stressed mice. Overall, our data indicate that mitochondrial dynamics pathways are implicated in the regulation of stress predisposition and responses in high anxiety.

P30

Which are the behavioral and mitochondrial correlates of early handling?

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Early life manipulations are studied in rodents to elucidate the persistent effects of early life events in adulthood. Early Handling (EH) is an early life manipulation where pups are briefly and repeatedly separated from their mother in early postnatal life. Using high (HAB) and normal (NAB) anxiety-related behavior mice, we investigated EH effects on maternal behavior, and on the behavioral and molecular profiles of male and female adult pups. Maternal behavior was observed in HAB and NAB mice dams from postnatal day 2 to 7. A behavioral test battery (social preference-avoidance test, dark-light box test, open field test, forced-swim test) was used to assess EH effects on HAB and NAB male and female pup behavior. At the molecular level, we used Western blots and biochemical assays to detect changes in mitochondrial housekeeping and metabolic functions. We also used real-time qPCRs to examine mRNA level alterations in mitochondrial dynamics, such as biogenesis, fission, fusion and mitophagy. Our results show that while EH did not affect maternal behavior within the HAB and NAB lines, significant differences were observed between the basal (non-handling group, NH) HAB and NAB dams. Interestingly, EH exerted anxiolytic effects in the dark-light box test in HAB-EH vs. HAB-NH male pups. The subsequent molecular analysis of HAB-EH vs. HAB-NH male mice, revealed that EH resulted in mRNA level alterations of key players of mitochondrial dynamics machinery in the prefrontal cortex of HAB male pups. Furthermore, prefrontal cortex and hippocampus protein levels and oxidative stress-related readouts correlate with behavioral parameters in HAB-EH mice. Overall, these findings highlight an implication of mitochondrial dynamics in EH-induced anxiolytic effects in HAB male mice. Follow up studies on the interplay of these EH-anxiolytic effects and brain mitochondria would facilitate discovery of candidate biomarkers and therapeutic targets for anxiety disorders.

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P31

Stable expression of SARS CoV-2-E protein in HEK-293 cells, study of its localization and investigation of its biological function

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At the end of 2019, the entire planet came face to face with a virus, which has so far infected 317 million people and caused around 5.5 million deaths. This virus is called SARS-CoV-2 and the disease it causes, COVID-19. The virus may lead to the overloading of the immune system through the activation of multiple cytokine cascades. One of the viral proteins responsible for initiating these cascades is protein E that is present in the envelope of the viral particle and takes part in its assembly and budding from the ERGIC (Breitinger et al., 2021). Moreover, E protein appears to function as viroporin, which has the ability to form membrane ion channels.

The purpose of this project was to study the biological activity of E protein in living cells. As a first step, HEK-293 cells stably overexpressing the SARS-CoV-2-E protein were generated by the use of a lentivirus-based plasmid. This construct was instructing the cells to overexpress the E protein, tagged with the sequence of two streptavidin tags (WSHPQFEK) at the C-terminus. Several HEK-293 clones were generated, that expressed the gene encoding the SARS-CoV-2-E-strep at the mRNA level. The expression of the strep-tagged E protein was confirmed by Western Blot using a commercially available antibody specific for streptavidin tags (Strep II Tag, Novus Bio). Next, we investigated the subcellular location and the topology of the SARS-CoV-2-E strep-tagged protein by subcellular biochemical fractionation and immunofluorescence. Preliminary data indicate that the SARS-CoV-2-E protein localizes predominantly in the Golgi, while a significant amount is also found in the cell plasma membrane. Using electrophysiological techniques, we are currently testing the ability of the E protein to function as viroporin.

Bibliography

Breitinger, U., Ali, N. K. M., Sticht, H., & Breitinger, H. G. (2021). Inhibition of SARS CoV Envelope Protein by Flavonoids and Classical Viroporin Inhibitors. *Frontiers in Microbiology*, 12(July), 1–14. <https://doi.org/10.3389/fmicb.2021.692423>

P32

Investigating the oncogenic role of the lncRNA Gracile2 in gastric cancer

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Gastric cancer ranks fifth in prevalence and second in mortality on a global scale. The ranking difference between frequency and mortality is due to delayed diagnosis since early stages of the disease are usually asymptomatic. Early diagnosis of gastric cancer relies mainly on gastroscopy, which is unsuited for routine monitoring of the population on a large-scale. Therefore, development of novel and specialized markers for early and non-invasive diagnosis and therapy is imperative. LncRNAs refer to regulatory non-coding transcripts that are involved in multiple aspects of physiology and pathology, often in a tissue- or cancer-specific manner, and therefore are regarded as valuable diagnostic and therapeutic agents. During our study, we focus on the characterization of GrACILe2, a lncRNA that is over-expressed in all stages of gastric cancer and its expression levels correlate with the patient survival. We evaluated the half-life time of the lncRNA transcript, as well as its subcellular localization in gastric cancer cells. Additionally, we performed a lenti-viral mediated knock-down of GrACILe2, linked its downregulation with a growth defect phenotype and proceeded to RNA-seq to detect possible downstream target genes that are directly or indirectly targeted by GrACILe2 function in the background of gastric cancer cells.

P33

Identification of compounds derived from the Greek flora with anti-aggregation properties

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Protein homeostasis or proteostasis refers to the molecular mechanisms that are responsible for the maintenance of the cellular protein network. Proteostatic mechanisms tend to deteriorate with age thus often leading to accumulation of toxic protein aggregates. The A β peptide that has been causally related to Alzheimer's disease (AD) onset and progression represents one of these aggregation-prone proteins. Plant secondary metabolites have been shown to be beneficial for the maintenance of protein homeostasis and/or its restoration. We have thus screened for natural products with anti-aggregating properties from the Greek flora, taking advantage of multiple *C. elegans* AD models. We have identified a mountain tea extract with anti-aggregation properties derived from the Greek endemic *Sideritis clandestina* subsp. *Peloponnesiaca* (SCP). We have further fractionated the extract to identify the specific bioactive compounds that are responsible for these properties. We show that the identified compounds may decelerate: (1) the progression of the AD phenotype in CL4176 nematode strain, a strain that conditionally expresses the human A β ₁₋₄₂ in its body wall muscle cells and undergoes paralysis due to A β aggregation, as well as, (2) the accumulation of A β aggregates in CL2331 nematode strain, a strain that expresses the human A β ₃₋₄₂ peptide fused to green fluorescent protein (GFP) in its body wall muscle cells where A β aggregates can be visualized in vivo. Our study uncovers the need for identification and characterization of bioactive compounds that ideally are part of our diet with anti-aggregation properties.

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P34

Profiling of ribosome constituents to molecularly unveil pharmacological and therapeutic maps for ribosomopathies

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Ribosomopathies comprise a diverse subset of human disorders in which the deregulation of ribosome biogenesis, translation, and mutational status of ribosomal proteins (RPs) has been postulated to contribute to the clinical phenotype. Since erythropoiesis is characterized by a developmentally regulated reduction of ribosomes number in a well-orchestrated regulation of gene expression, the application of well-established erythropoiesis cell models is considered of crucial pharmacological and clinical importance to unveil molecular causality features of ribosomopathies including cancer susceptibility. To this end, by applying multi-omics methodologies and suitable cellular erythropoiesis models, our recent work has provided new insights to ribosomopathies since: a) Based on extensive sequence comparisons and systematic curation, it has been established a reference set for RPs in eleven vertebrate species, having quantified their sequence conservation levels and correlated their coordinated gene expression patterns to assess tissue specificity; b) Ribosome protein stoichiometry in isolated ribosomes upon the course of erythroid maturation has been analyzed using the established model of murine erythroleukemia cells (MEL) by applying quantitative proteomics. The obtained data suggest that: a) RPs exhibit a complex relationship between their structure and function that broadly maintains a consistent expression landscape across tissues, while most of the variation arises from species idiosyncrasies. b) The variation seen in the tissue-specific gene expression patterns of RPs is rather species-related and not due to tissue-based evolutionary processes. This fact was confirmed by analyzing the gene expression patterns in the 11 vertebrate species and within up to 33 tissues and it was also validated by the development and application of machine learning models. and c) Upon erythroid differentiation the extensive reprogramming of the overall ribosomal levels was characterized by an increase in monosomes and a decrease in polysomes, without, however, any change in the main ribosomal architecture that remained invariable between immature and differentiated cells.

P35

Differential effects of human Tau isoforms to neuronal dysfunction and toxicity in the Drosophila CNS

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Accumulation of highly post-translationally modified Tau proteins is a hallmark of neurodegenerative disorders known as Tauopathies, the most common of which is Alzheimer's disease. Although six Tau isoforms are found in the human brain, the majority of animal and cellular Tauopathy models utilize a representative single isoform. However, the six human Tau isoforms present overlapping but distinct distribution in the brain and are differentially involved in particular Tauopathies. These observations support the notion that Tau isoforms possess distinct functional properties important for both physiology and pathology. To address this hypothesis the six human brain Tau isoforms were expressed singly in the Drosophila brain, and their effects in an established battery of assays measuring neuronal dysfunction, vulnerability to oxidative stress and life span were systematically assessed comparatively. The results reveal isoform-specific effects clearly not attributed to differences in expression levels but correlated with the number of microtubule-binding repeats and the accumulation of a particular isoform in support of the functional differentiation of these Tau isoforms. Delineation of isoform-specific effects is essential to understand the apparent differential involvement of each Tau isoform in Tauopathies and their contribution to neuronal dysfunction and toxicity.

P36

Studying SLC25A46-mediated pathogenic mechanisms in a genetic mouse model of neuropathology with proteomic analysis

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SLC25A46, a member of the Solute Carrier 25 (SLC25) family of nuclear-encoded mitochondrial transporters, has been recently identified as a novel genetic cause of a wide spectrum of human neurological diseases, such as optic atrophy, Charcot-Marie-Tooth type 2, Leigh syndrome, progressive myoclonic ataxia and pontocerebellar hypoplasia. Our group has recently identified a non-sense point mutation in the mouse *Slc25a46* gene, resulting in a truncated protein, that causes lethal neuropathology in mice (*Slc25a46^{atc/atc}*), characterized by ataxia, optic atrophy and cerebellar hypoplasia, similarly to human pathology. SLC25A46 localizes in the outer mitochondrial membrane and so far there is no evidence for transport activity. Recent studies have shown involvement of SLC25A46 in cristae maintenance and mitochondrial dynamics, through interaction with core MICOS complex subunits MIC60 and MIC19 and mitochondrial fusion proteins OPA1 and MFN1/2. In addition, our group recently showed that SLC25A46 interacts with a large number of proteins and protein complexes involved in the mitochondria architecture, energy production, and flux and also in inter-organellar contacts.

Our present work focuses on the investigation of the pathogenic mechanisms involved in the SLC25A46-mediated neuropathologies. Towards this scope, a comparative proteomic approach was performed in whole-cell extracts isolated from both the cerebellum of *Slc25a46^{atc/atc}* mice and WT littermates and a shRNA-mediated *Slc25a46* knockdown HEK-293 cell line using LC-MS/MS. Proteomic analysis in whole-cell extracts from both *Slc25a46^{atc/atc}* cerebellum and *Slc25a46* knockdown cells revealed a notable reduction in proteins participating to the respiratory electron transport chain of oxidative phosphorylation, implying impaired mitochondria function. Also, our results support the involvement of SLC25A46 in mitochondrial dynamics, as proteins crucial for the fusion of inner mitochondrial membrane such as OPA1, were more abundant in *Slc25a46^{atc/atc}* mice. Furthermore, members of the MICOS complex, including IMMT and CHCHD3 involved in the mitochondrial cristae maintenance, were increased in SLC25A46-deficient extracts.

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P37

Functional characterization of long non-coding RNAs with CRISPR/Cas9 approaches in gastric cancer

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Gastric cancer (GC) ranks fifth in incidence and third in mortality worldwide. Histological and molecular criteria categorized this malignancy into several subtypes, highlighting the strong heterogeneity that is typically observed among patients. The high mortality rates reflect the delayed diagnosis of the disease, due to asymptomatic early stages. Current diagnostic protocols rely mainly on invasive gastroscopy, while the existing molecular biomarkers lack specificity and sensitivity. Thus, it is imperative to develop novel, non-invasive molecular biomarkers for the early and specific detection of gastric cancer. Long non-coding RNAs (lncRNAs) can serve as ideal biomarkers due to their highly specific expression patterns and function. LncRNAs are functional transcripts larger than 200nt with low or no coding potential. Their tissue and/or cancer-specific expression ensures high functional specialization both in physiological and cancer-related processes, such as cell proliferation, angiogenesis, apoptosis, and metastasis. Additionally, lncRNAs serve as ideal non-invasive biomarkers inasmuch they can be accurately detected in circulating fluids of patients. The aim of this study is the development and optimization of various CRISPR/Cas9 methodologies to functional characterize lncRNAs in the context of gastric cancer initiation and progression. To this end, an optimized lentiviral transduction system was used to develop molecular tools like CRISPR activation (SAM system used for overexpression) and CRISPR inhibition (KRAB_MeCP2 system used for knock-down) to study lncRNA phenotypic and molecular impact following their cis-overexpression or epigenetic silencing. In parallel, a CRISPR/Cas9-mediated knock-out approach was also developed to challenge cancer cell growth after complete impairment of lncRNA function. Collectively, these approaches provide us with an expanding molecular toolbox to evaluate lncRNA impact at the phenotypic and molecular level, with the aim of expanding the diagnostic toolbox of the disease with novel and highly specific molecular biomarkers.

P38

Evaluation of metabolic outcomes of *Crh*^{-/-} mice exposed to diet-induced obesity

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Introduction

Nowadays, there is an increasing rate of western-type diet consumption, consisting predominantly of fat, which in time can lead to obesity. Moreover, chronic stress exposure results in Hypothalamic-Pituitary-Adrenal (HPA) axis over-activation which further disrupts the organism's energy homeostasis state. Previous work has shown the contradictory effects of glucocorticoid, the end-product of HPA axis, in metabolism, but the metabolic outcomes of diet-induced obesity in relation to overall alterations in HPA-axis activity has not been investigated. As Corticotropin-Releasing-Hormone (CRH) is the leading neuropeptide for the activation of HPA axis, the aim of the present study was to investigate the metabolic effects of diet-induced obesity in mice lacking CRH (*Crh*^{-/-}).

Materials-Methods

Crh^{-/-} and wild-type (*Crh*^{+/+}) mice were fed with high-fat (HFD45%) or standard-chow (SC) diet for 13 weeks. Weight-change and food-intake were measured weekly. Blood glucose levels were measured in the fasting state before and after the 13-weeks' feeding. Glucose-tolerance-test (GTT) was performed via i.p. dextrose injection after the 13-weeks period. Mice were sacrificed and White-Adipose-Tissue (WAT) was collected for qPCR analysis.

Results

Fasting blood glucose levels were dramatically increased in HFD45%-fed compared to SC-fed mice regardless of genotype. Wild-type mice gained significantly more weight when fed with HFD45% compared to *Crh*^{-/-} mice although the amount of HFD45% food-intake was the same between genotypes. Additionally, *Crh*^{-/-} mice were capable of faster glucose clearance measured by GTT than wild-type mice in the HFD45% group. Interestingly, leptin mRNA levels were significantly less in WAT of *Crh*^{-/-} mice compared to wild-type mice in the HFD45% group.

Conclusions

Our results provide evidence that *Crh*^{-/-} mice have improved metabolic outcomes when exposed to diet-induced obesity. Interestingly, the *Crh*^{-/-} mice exhibited reduced weight gain and improved glucose homeostasis under diet-induced obesity compared to wild-type mice. Our ongoing analyses attempt to further elucidate the metabolic mechanisms of this phenomenon.

P39

Relation of WW domain-containing E3 ubiquitin-protein ligase 1 (WWP1) overexpression and cancer hallmarks

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WW domain-containing E3 ubiquitin-protein ligase 1 (WWP1) belongs to the C2-WW-HECT E3 ligase family, ubiquitinates a variety of substrates, and regulates diverse cellular processes. Thus, WWP1 dysregulation is involved in various diseases, such as malignancies, cardiovascular diseases, and immune disorders. Vast evidence reveals that WWP1 is overexpressed in multiple cancers, such as breast, colon, and prostate, as well as, in melanoma. Polyubiquitination by the WWP1 enzyme suppresses the dimerization, membrane recruitment, and function of PTEN.

The DNA sequence of WWP1 was amplified from MCF-7 breast cancer cells and cloned in pT7-IRES His-C DNA vector for the *in vitro* transcription of WWP1 mRNA and consequently, transfection of the PTEN-deficient cancer cell line, ZR-75-1, was performed. RT-qPCR and Western blot analysis for the recombinant His-Tag WWP1 protein revealed the successful transfection of ZR-75-1 and significant differences in markers related to cancer hallmarks were found. At the mRNA level, a substantial increase of PCNA following a reduction of p21 confirms the possible involvement of WWP1 in proliferative signaling. In contrast, the increased transcripts ratio of Bax/Bcl-2 indicated that WWP1 exerted a probable anti-apoptosis role in breast cancer cells. Additionally, the decrease of E-cadherin expression in conjunction with an increase in MMP-1, -2, and -9 supports the evidence that WWP1 may influence the EMT program as well as the complex processes of invasion and metastasis. Simultaneously, an elevated angiogenesis potential was found based on mRNA expression levels of VEGFA as well as an increased expression of endoplasmic reticulum stress markers, such as ATF4 and CHOP. To conclude, WWP1 overexpression satisfies almost all cancer hallmarks *in vitro* and, as a result, targeting WWP1 protein could be of therapeutic potential and holds promise for patients affected by a number of cancers and other disorders.

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Aggregation studies on peptide-analogues of human Serum Amyloid P component

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Serum Amyloid P component (SAP), or 9.5S alpha-1-glycoprotein, is a serum glycoprotein whose structure has been determined at high resolution, but its physiological function has yet to be found. SAP is widely known for its association with amyloidoses, since it binds with amyloid fibrils in a calcium-dependent way and is present in most cases of amyloid deposits. It is proposed that SAP takes part in the coating and consequently protection of amyloid fibrils from the cellular mechanisms that recognize, and then remove, these abnormal structures. However, it is not clear whether SAP actively participates in the formation of amyloid fibrils *in vivo*. To clarify its potential implication in amyloid formation, we located aggregation-prone regions within the mature sequence of SAP, with the aid of AmylPred2. AmylPred2 is a consensus algorithm developed by our laboratory that combines 11 independent computational methods, in order to find regions with high aggregation propensity. The predicted regions were chemically synthesized and studied by applying molecular biophysical techniques, such as Transmission Electron Microscopy, Congo Red birefringence assay, ThT fluorescence assays and ATR FT-IR Spectroscopy. Our experimental findings indicate that some peptide-analogues of SAP can form amyloid fibrils *in vitro*. Consequently, the possibility that the whole protein aggregates can not be eliminated.

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P41

Pipeline development for the mutational analysis of beta-thalassemia patients

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Beta thalassemia is a hereditary blood disorder characterized by abnormal synthesis of hemoglobin beta chains, which can either be absent (β^0) or reduced (β^+). It is an autosomal recessive disease with variable phenotypes ranging from severe anemia (thalassemia major, intermedia) to clinically asymptomatic individuals (carrier state). Molecularly, β -thalassemia is very heterogeneous, with more than 200 disease-causing mutations having been identified until now. Most of them include single nucleotide substitutions, deletions, or insertions, while gross gene deletions are rare¹ to a clinically asymptomatic state exemplifies how a spectrum of disease severity can be generated in single gene disorders. While the genetic basis for β thalassemia, and how severity of the anemia could be modified at different levels of its pathophysiology have been well documented, therapy remains largely supportive with bone marrow transplant being the only cure. Identification of the genetic variants modifying fetal hemoglobin (HbF. The incidence of β -thalassemia trait in the broader Mediterranean area is estimated to be 7-8%, considered to be high compared to the rest of the European Union^{2,3} which is observed in the broader Mediterranean area has led to the establishment of molecular diagnostics' assays to prevent affected births. Therefore, the development of a reliable, cost-effective and rapid scanning method for β globin gene point mutations, easily adapted to a routine laboratory, is absolutely essential. Here, we describe, for the first time, the development of a High-Resolution Melting Analysis (HRMA. Therefore, a reliable, cost-effective, and rapid scanning method for the beta globin gene point mutations, is necessary. The phenotype of beta-thalassemia can be modified by factors mapping outside the globin gene cluster, highlighting the need of scanning the patients' genome¹ to a clinically asymptomatic state exemplifies how a spectrum of disease severity can be generated in single gene disorders. While the genetic basis for β thalassemia, and how severity of the anemia could be modified at different levels of its pathophysiology have been well documented, therapy remains largely supportive with bone marrow transplant being the only cure. Identification of the genetic variants modifying fetal hemoglobin (HbF. Here, we developed an assay for the mutational analysis of the beta globin gene for thalassemic patients, which could easily be adapted to a clinical laboratory. It includes the amplification of the β -globin gene via polymerase chain reaction (PCR), followed by Sanger sequencing analysis. The pipeline for sequencing analysis filters the variants based on the quality of the sequencing and the number of reads. The detected variants are annotated and called based on the ClinVar database, however novel variants can be unveiled too. In the future, this pipeline could easily be adapted for analysis of multiple genomic regions, assisting to map possible mutations outside the beta globin locus.

¹ Thein, S. L. Molecular basis of β thalassemia and potential therapeutic targets. *Blood Cells, Mol. Dis.* 70, 54–65 (2018).
² Chassanidis, C., Boutou, E., Voskaridou, E. & Balassopoulou, A. Development of a high-resolution melting approach for scanning beta globin gene point mutations in the Greek and other mediterranean populations. *PLoS One* 11, 1–22 (2016).
³ Papachatzopoulou, A. et al. Region-specific genetic heterogeneity of HBB mutation distribution in South-Western Greece. *Hemoglobin* 34, 333–342 (2010).

P42

Identification of potential new targets in chemoresistant triple negative breast cancer cells through metabolomic analysis

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Drug resistance is the greatest hurdle for successful therapeutic results and is associated with most cancer deaths. In triple negative breast cancer (TNBC), where there is a lack of specific therapeutic targets, conventional chemotherapy consists of the frontline treatment. TNBC patients initially respond well to chemotherapy, but they eventually develop drug-resistance and subsequently relapse and metastasis 3-5 years after diagnosis. Metabolic reprogramming, one of the hallmarks of cancer, is involved in the development of drug-resistance. In this study, we conducted metabolomic and lipidomic analysis in paclitaxel-resistant TNBC cells and their non-resistant counterparts (SUM159 PTX-res and parental cells, respectively) for elucidating the metabolic pathways that are involved in drug-resistance. For metabolite and lipid extraction from both cell lines we used a mixture of methanol, chloroform and water in a ratio of 1:1:0.33 (v/v/v) for the generation of a dual-phase procedure. Polar metabolites and non-polar lipids were contained in the methanol/water phase and the chloroform phase, respectively. The cellular extracts were processed using ¹H-NMR spectroscopy. NMR spectra were further analysed using the Chenomx software for the identification and quantification of the intracellular metabolites and lipids. Further analysis of the overall metabolic and lipidomic profiles was conducted using the Metaboanalyst 5.0 software. The results of our work revealed numerous metabolites and lipids that were significantly altered in the PTX-res cells compared to parental cells, while the analysis of the overall metabolic and lipidomic profiles showed a sharp separation that distinct the two cell lines. The separation of the metabolic profiles was mainly attributed to myo-inositol, a cell membrane compound that was significantly lower in the PTX-res cells. Moreover, the lipidomic profile analysis highlighted the role of the cholesterol biosynthesis pathway as the main upregulated pathway in the SUM159 PTX-res cells. Overall, our data provide a critical insight into the metabolic adaptations that accompany acquired resistance in TNBC pinpointing potential new targets.

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Methylation imprinting of *MIR125B1* promoter as potential predictor in pediatric acute lymphoblastic leukaemia prognostication

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Acute lymphoblastic leukaemia (ALL) represents the most frequently diagnosed malignancy in children, constituting ~25% of all pediatric cancers worldwide. Despite the marked reduction in disease-specific mortality over the last decades, a significant number of patients present resistance to antileukaemic agents and dismal prognosis. In this regard, the identification of novel molecular markers could tackle with the risk for high toxicity phenomena, ameliorating response to therapeutic protocols and disease relapse monitoring. Our previously published findings revealed that reduced levels of miR-125b are associated with poor survival outcome, while lncRNA MIR100HG indicates oncogenic role in other hematological malignancies, prompting us to study the methylation status and clinical value of a region which embedded *MIR125B1* and *MIR100HG* regulatory elements. Genomic DNA (gDNA) was extracted from 52 bone marrow (BM) specimens. gDNA underwent bisulfide conversion, followed by PCR amplification of specific CpGs in weak and active enhancers of *MIR125B1* distal promoter (hosted into *MIR100HG* intron). Quantification of methylation levels was accomplished by pyrosequencing of the PCR products via PyroMark Q24 platform. Progression-free survival (PFS) and overall survival (OS) were used to assess the survival outcome of chALL patients. The chALL samples displayed elevated methylation levels of all studied CpG loci compared to healthy individuals ($p < 0.001$), along with an upward trend from weak [26.5%±2.3(3.7-69.2%)] to active enhancer [54.8%±2.7(10.6-81.3%)]. Importantly, the loss of *MIR125B1* methylation in active enhancer was correlated with unfavorable prognosis traits, namely increased age (>10 years old; $p = 0.043$) and absence of t(12;21)(p12;q22)/TEL-AML1 translocation ($p < 0.001$). Ultimately, survival analysis unveiled that loss of *MIR125B1* methylation in active enhancer was related to significantly shorter OS (log-rank $p = 0.032$) and inferior PFS (log-rank $p = 0.006$). Overall, significantly elevated methylation was highlighted for all studied CpG sites in chALL patients compared to healthy cases, while diminished levels of *MIR125B1* distal promoter methylation improves the prognosis of chALL patients' outcome.

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Molecular Components of Protein Synthesis-Independent Memory in *Drosophila*

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Understanding the molecular mechanisms that govern memory will most likely inform methods to combat memory disorders including dementias and age-dependent cognitive impairments. Learning and memory are studied in mammalian and simpler model organisms, including *Drosophila melanogaster*. Interestingly, flies are able to form a memory type which unlike typical Long-Term Memories is independent of protein synthesis (PSIM). Although PSIM has been systematically studied in *Drosophila*, it is unlikely that it has not been conserved through evolution and in fact, -PSIM characteristics have been identified in other invertebrates and vertebrates including humans. Because the mechanisms that govern PSIM are largely unknown, we used the massed conditioning (MC) method to reveal molecular components of this novel memory in *Drosophila*. We capitalized on our characterization of the adaptor protein Drk, which acts downstream of tyrosine-kinase receptors and is essential in the Mushroom Bodies (MBs), -the center of learning and memory in the fly- for normal MC-elicited memory. Using MB-limited proteomic approaches we identified a number of Drk-interacting proteins, including novel molecules that might participate in the same signaling pathway as Drk and are engaged in PSIM. We report on the functional validation of these Drk-interacting proteins, which supports roles for a number of them in MC-elicited memory. Further study of their function and signaling will hopefully aid our understanding of the mechanisms that underlie protein synthesis-independent memories not only in *Drosophila*, but also in mammals.

P45

Identification of a small molecule inhibitor of hyaluronan synthesis, DDIT, targeting breast cancer cells

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Breast cancer is a highly heterogenous disease and one of the most common cancers in women. Breast cancer cells synthesize large amounts of hyaluronan to assist their proliferation, survival, migration and invasion.

Currently, the only known small molecule inhibitor of hyaluronan synthesis is 4-methyl-umbelliferone (4-MU). Due to the importance of hyaluronan for breast cancer, our aim was to identify more potent inhibitors of its synthesis.

Upon screening a list of candidate compounds, we identified one compound (DDIT) as a potent hyaluronan synthesis inhibitor in several cell types. Further investigation of this compound suggests that is non-toxic and more potent than 4-MU. Treatment of Hs578T triple-negative breast cancer cells with DDIT reduced the expressions of the main hyaluronidase TMEM2 and hyaluronan receptors CD44s and RHAMM. On the other hand, no effect was evident in the expression of hyaluronan synthases. Upon further investigations, we found that DDIT reduced breast cancer cell proliferation by arresting cells in G0/G1 phase, as evidenced by FACS analysis. DDIT was also able to reduce migration of breast cancer cells in normal lung microenvironment. Importantly, DDIT abrogated breast cancer stem cell self-renewal and reduced the expression of TMEM2 in cells grown in low-attachment conditions.

Collectively, the novel compound DDIT seems to be a promising small molecule for breast cancer treatment through modulation of hyaluronan synthesis.

P46

The effects of human recombinant thyrotropin on cell proliferation and migration. A study on normal and cancerous thyroid cell lines

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Thyrotropin (TSH) is considered a mitogen for thyroid cells thus TSH suppression is required in the management of patients with Papillary Thyroid Carcinoma (PTC). However, recent data suggest that the molecular alterations implicated in thyroid tumorigenesis are mainly the activation of receptor tyrosine kinase (RTK) pathways, such as the mitogen-activated protein kinase (MAPK) and the phosphoinositide 3 kinase (PI3K), and the role of TSH is overlooked. If this is true, then a substantial number of PTC patients suffer from iatrogenic hyperthyroidism needlessly (via negative feedback inhibition of TSH secretion). To study this, the thyroid cell line Nthy Ori 3-1 is utilized as a normal thyrocyte model and the cancer cell lines K1 and TPC-1 are used to monitor the effects of TSH on carcinoma cells. We used recombinant human TSH (rh-TSH) and not bovine TSH (b-TSH) used in previous studies as it is expected to mimic better the conditions inside the human body. In this study, the hormone is administered either alone or with the presence of insulin to monitor these synergistic effects. To multiply the effects of the hormone, cell clones (from the three cell lines) that stably overexpress the TSH receptor were prepared using a lentiviral vector/Tet operon system, and the increased expression levels were verified both at the RNA and protein levels. No significant changes in the tested parameters were detected disproving the role of TSH as a mitogenic agent without the synergistic function of other signaling molecules.

P47

The effects of Polyunsaturated Fatty Acids on cancer cell lines; A proliferation and migration study

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The anticancer effects of many Polyunsaturated Fatty Acids (PUFAs) is a not widely researched field, due to the lack of adequate quantities and ways to specifically administrate them to cancer patients and monitor their effects. The two large families of PUFAs, the ω -3 and ω -6 group of fatty acids, are considered essential parts of the human diet because of our incapability to biosynthesize most of them. PUFAs are absorbed through our diet with nuts and legumes being rich in ω -6 fats and seafood containing high amounts of ω -3, produced by the phytoplankton and incorporated inside marine organisms. The development of bioreactors and the utilization of oleaginous fungi has allowed the bulk production of PUFA from low-cost substrates and has opened a new field of research with the potent use of PUFAs as anticancer medication. Some members of the PUFA family have been shown to induce cell death by increasing oxidative stress inside cells and interfering with cell signaling cascades. So far, PUFAs are linked to inflammatory responses and suppression of malignancies, however, the exact mechanisms are not fully understood, and unveiling their effects could offer new medication schemes and approaches to the fight against cancer. This study focuses on PUFA's effects on vital cancer cell functions like survival, proliferation, and migration, trying to shed light on the concertation, form, and way of administration that produce the best results and inhibit cell growth. The form of PUFAs used is an esterified form with Lithium ions (FALs) that are water-soluble.

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P48

Development of drug resistance against the proteasome inhibitor Bortezomib; Effects on main signaling pathways, apoptosis, and oxidative stress

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The proteasome inhibitor Bortezomib (Velcade®) is a drug administered to treat multiple myeloma and has potential use as a treatment against prostate cancer and other types of malignancies. Although it has many positive aspects exhibiting low toxicity and few side effects, the main disadvantage is the appearance of resistant cell populations that manage to survive and are usually more aggressive requiring stronger medications. Bortezomib acts by specifically binding to the $\beta 5$ subunit of the 26S proteasome and disrupts the protein degradation pathways leading to increased stress and eventually cell death by induction of apoptosis pathways. Cancer cells are more susceptible to proteasome disorganization due to the increased protein synthesis that leads to the accumulation of defective polypeptides. Within some weeks of administration phenomena of resistance are documented, mainly because of mutations at the *PSMB5* gene, the gene coding for the $\beta 5$ subunit, and the mutated clones have lower affinity for the drug as well as increased levels of 26S proteasome to stave off the effects of the drug. In this study, Bortezomib-resistant cell populations are created and subsequently, we try to uncover the phenotypic changes a resistant cell compiles. The main targets include survival, proliferation, migration, and apoptosis signaling pathways and response to oxidative stress caused by the drug, to understand better the differences between resistant and non-resistant cells on the prostate cancer cell lines DU-145. The differences between the two groups could act as therapeutic targets if a universal mechanism emerges.

P49

Administration of Carfilzomib at cell lines resistant to the proteasome inhibitor Bortezomib and studying the effects on main cell functions

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Carfilzomib (Kyprolis[®]) is a second-generation proteasome inhibitor utilized to treat multiple myeloma in patients that have been previously treated with Bortezomib and disease progression is observed due to drug resistance. Carfilzomib exhibits more side effects than Bortezomib and for that reason is always used as a second-line medication even though it is believed to have the same target inside cells, targeting the $\beta 5$ subunit of the 26S proteasome. By disrupting the $\beta 5$ subunit chymotrypsin-like activity, the proteasome is deactivated, and the protein degradation pathways are inextricably disrupted causing the accumulation of damaged proteins and eventually the apoptotic death of the cell that is unable to maintain its homeostasis. Cancer cells are more susceptible to the deregulation of this system due to the higher biosynthetic rates they have compared to normal cells. In this study, Carfilzomib is administered to different groups of the DU-145 cancer cell line; cells resistant to Bortezomib, cells resistant to Carfilzomib, cells that have acquired resistance to Carfilzomib after continuous growth with Bortezomib, and naïve cells, and the main differences on cell viability, proliferation, and migration rates are recorded. Carfilzomib has fewer off-target effects being more specific and safer than Bortezomib but the development of cross-resistance to both substances is to be expected so the unveiling of causing mechanisms will allow the improvement of the current therapeutic approach and the design of more safe and effective treatments.

P50

Evaluation of the role of synthetic neurosteroids in psoriasis

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Psoriasis is a chronic, inflammatory, autoimmune disease which pathogenesis remains unknown. Multiple genes, as well as environmental factors play a key role in triggering the disease. Psoriasis is characterized by alterations in the physiology of the skin and as a result, a wide range of immune cells, especially antigen presenting cells are accumulated and contribute to the initiation of the disease.

Clinical studies have shown an association of neurosteroid dehydroepiandrosterone (DHEA) with skin conditions. DHEA levels were found low in patients with psoriasis. However, the exogenous administration of DHEA is not beneficial due to the immediate metabolism into androgens and estrogens.

BNN27 is a synthetic analogue of DHEA with beneficial effects in *in vivo* and *in vitro* models of inflammation and immune diseases. Compared to DHEA, BNN27 metabolism is delayed. Thus, the scope of this study was to examine the role of the synthetic neurosteroid in psoriasis using an *in vivo* model of imiquimod-induced psoriasis in mice.

Methods: Male mice aged 8-12 weeks were used. Psoriasis was induced using imiquimod (IMQ). BNN27 in 100, 50, 10 mg/kg concentrations was injected intraperitoneally for 7 days. Mice were sacrificed, photos were taken, and blood and tissues were collected. The severity of psoriasis was assessed on the basis of PASI Score.

Results: BNN27-treated mice (10mg/Kg) showed significantly reduced PASI Score on the 6th day of treatment. Serum levels of IL-6 and IFN- γ of the same mice were also reduced. Skin IL-23A, IL-6, TNF- α , p75 and NGF mRNA levels were also decreased in BNN27 treated mice at the lowest concentration.

Conclusions: Our findings suggest that BNN27 may play an anti-inflammatory role in psoriasis in a dose-dependent manner. However, further study of cytokine and protein levels following treatment with BNN27 will be useful tools for the monitoring of the disease.

P51

ERK1/2 and JNK pathways regulate MMP-3 and MMP-9 induction during *Helicobacter pylori* infection.

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Persistent *Helicobacter pylori* (*Hp*) infection of human gastric mucosa is a primary risk factor for carcinogenesis. Expression of *Hp* virulence factor CagA contributes significantly to the severity of clinical outcome. CagA, upon its intracellular delivery by the bacterial Type 4 secretion system (T4SS) and subsequent tyrosine phosphorylation by host kinases, has been reported to deregulate multiple signaling pathways, thereby inducing cellular invasiveness and enhanced proteolytic activity in the extracellular matrix. Accordingly, in vitro infection with *Hp* strains has been shown to result in CagA phosphorylation-dependent MMP-3 and MMP-9 aberrant expression, in line to the appearance of a characteristic elongation and scattering phenotype. We extended these findings by inoculating C57BL/6 mice with 3 different *Hp* strains, namely HPARE, a CagA-positive strain, HPARE Δ CagA mutant and SS1, a CagA-positive yet T4SS defective strain. Following 6 and 9 months of infection, MMP-3 was found transcriptionally upregulated in murine stomachs, relative to CagA expression. Concomitantly, immunohistochemical overexpression of MMP-3 and MMP-9 protein was determined in *Hp*-infected gastric mucosa. We further dissected contribution of MAPK pathways in the induction of MMP-3 and MMP-9, during 24h *Hp* infection of gastric epithelial cell lines AGS and GES-1, in the presence of chemical inhibitors of JNK (SP600125), ERK1/2 (PD98059) and p38 (SB203580) pathways. Although *Hp* infection alone induced elevated mRNA and protein levels of both MMPs, accompanying inhibition of ERK1/2 resulted in reduced mRNA and protein expression of MMP-3 and MMP-9, in both cell lines. Likewise, JNK inhibition resulted in downregulation of both MMPs in GES-1 cells. Interestingly, upon p38 inhibition in *Hp*-infected GES-1 both proteases remained transcriptionally and translationally unaltered. Our data indicate that ERK1/2 and JNK pathways regulate MMP-3 and MMP-9 induction possibly involving a crosstalk between MAPK pathways.

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P52

Association of VEGF-A and VEGFR-2 gene polymorphisms with age-related macular degeneration in a Southwest Greek population sample

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Age-Related Macular Degeneration (AMD) is one of the leading causes of blindness in people over 60y, appearing more frequently in the Caucasian population and causing gradual loss of central vision.

Among the factors that cause AMD, studies have singled out the most important ones: age, smoking, hypertension, diet, race, and genetic susceptibility of patients.

Regarding genetic factors, the most important studies are based on the association of various polymorphisms (SNPs) in complement system genes (C2, C3, C9, CFH, CFB, CFI, and MBL) with AMD.

Although the pathology of AMD is still under investigation, recent studies have shown that vascular endothelial growth factor (VEGF) is associated with the likelihood of developing the disease. The family of VEGF proteins and their membrane endothelial receptors (VEGFR) play an important role in the process of angiogenesis.

The present study refers to a population of southwestern Greece and deals with the association of SNPs rs1413711 and rs2071559 of the VEGF-A and VEGFR-2 gene, respectively, with the appearance risk of AMD. The study involved 78 patients with AMD and 63 healthy controls for rs1413711 and 74 patients with AMD and 74 healthy controls for rs2071559, who visited the Ophthalmology Clinic of the University General Hospital of Patras. All participants were genotyped for the respective polymorphisms, using an RFLP-PCR assay.

According to the results of the present study, homozygosity (TT) of the rs1413711 SNP but also the homozygosity of the normal allelic (CC) did not occur with a significant deviation in controls and patients. Regarding the rs2071559 SNP, homozygosity (TT) was found with a higher frequency in the sample of healthy people, while the sample of patients showed a higher percentage of heterozygotes (CT). At the same time, for both SNPs, smoking seems to contribute to the appearance of a pathological phenotype, in contrast to hypertension and family history.

P53

Chios Mastic extract supplementation alters Proteostatic mechanisms and possesses cardioprotective properties

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Chios mastic (CM) (*Pistacia lentiscus* var. *Chia*) is one of the oldest phytotherapeutics of purely Greek origin; it also has a Protected Designation of Origin based on EU Regulation No. 123/1997. In particular, several valid studies highlight CM antioxidant, antibacterial, antifungal, anticancer, anti-inflammatory, and hypolipidemic effects. Nonetheless little is known about the effect of CM on blood pressure and arterial aging. Hypertension (a major cause of death globally) and aging are characterized by vascular remodeling as well as endothelial dysfunction. It has been shown that the proteome functionality control network (Proteostasis Network) declines with aging and its dysfunction have been associated with cardiovascular complications (e.g., heart failure, ischemic syndromes, and/or hypertension). At first, we developed a robust, reliable, and simple HPLC-ELSD methodology to quantify masticadienonic and isomasticadienonic acids, CM's major and most characteristic triterpenes for the authentication and quality control of marketed CM-based products. During the Hyper-Mastic project, we also aim to study the effect of CM extracts and isolated pure molecules on the cardiovascular system by conducting a randomized clinical study on hypertensive patients. To this end, the anti-oxidative activity, and the effect on the vascular function of CM extracts are evaluated in the HUVEC cell line as well as in isolated blood cells of patients with hypertension. Our preliminary data suggest that treatment of cells with CM extracts alters proteostatic responses (Ubiquitin-Proteasome Pathway, Autophagy-Lysosome Pathway, and ROS production), indicating an enhancement in the functionality of protein degradation machineries and the antioxidant responses. In accordance with our cell-based findings, the administration of CM supplement activates proteostatic mechanisms in patients with hypertension. Overall, our preliminary findings support the cardioprotective effects of CM and propose Mastic as a potential pharmacological agent against hypertension.

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P54

The role of platelets on postnatal brain Neural Stem and Progenitor Cells of the Subependymal Zone niche before and after demyelination in the corpus callosum

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In the postnatal mammalian brain Neural Stem and Progenitor Cells (NSPCs) are located within specialized microenvironments, called stem cell niches, one of which is the Subependymal Zone (SEZ) of the lateral walls of the lateral ventricles. Previous in vivo work has revealed that focal demyelination in the adjacent corpus callosum (CC) affects the SEZ, increasing the number of proliferating SEZ-resident NSPCs, an effect that is correlated with the specific aggregation of platelets in the niche vasculature (1). In addition, using a coculture assay of platelets and NSPCs, it has been demonstrated that platelets affect the behaviour of the latter in differentiation and proliferation conditions. Here, we investigate further if these effects are due to direct cell-to-cell interactions, performing cocultures, as well as NSPC cultures in medium that has been enriched with platelet factors (conditioned medium). Preliminary results reveal that in the presence of high platelet densities the percentage of Sox2 immunopositive NSPCs increases in differentiation conditions, an effect that is not repeated when cultured in conditioned medium, reinforcing the role of cell-to-cell interactions between platelets and NSPCs. We also extend our in vivo analysis, performing experiments of focal demyelinating lesion on the CC in animals with reduced numbers of platelets, using two different procedures: transient chemical platelet depletion, as well as thrombocytopenic Nbeal2 knockout mice that have thrombocytopenia and platelets that are lacking functional α -granules (functional thrombocytopenia).



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References

¹ Kazanis, I., Feichtner, M., et al., Rivera, F. J. (2015). Lesion-induced accumulation of platelets promotes survival of adult neural stem/progenitor cells. *Experimental neurology*, 269, 75-89

P55

Generation of human pluripotent stem cells derived retinal Neuro-Vascular Unit as a model to study retinal diseases

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Retinal diseases such as diabetic retinopathy and age-related macular degeneration are the major causes of blindness nowadays. It has been recently shown that the dysfunction in the relationship between the neuroretina and the vascular system (neurovascular unit- NVU) plays a crucial role in the pathophysiology of these diseases. Thus, the advancement of reliable experimental model systems in order to study the NVU is an urgent need and the generation of human retinal organoids (ROs) is the ideal approach to do this given the limitations of the use of experimental animal models. However, ROs that have been generated and differentiated from pluripotent stem cells (PSCs) lack vascularization and thus their maturation is impaired. Consequently, these organoids cannot represent the NVU successfully. Our work is focused on the generation of ROs from human PSCs consisting of both neuronal and vascular cells (endothelial and mural cells- ECs, MCs). We have already generated and extensively characterized ECs and MCs derived from hPSCs. hPSCs derived ROs are currently generated and characterized using a sequential step strategy, mimicking the spatio-temporal development of the retina in vivo. Our plan is to vascularize these ROs in order to develop the retinal NVU (rNVU) in the best anatomical layout. Furthermore, our in vitro rNVU will serve as a model to elucidate the pathophysiology of Retinitis Pigmentosa (RP) (an inherited disease causing blindness) using patient-derived iPSCs with a PRPF31 mutation, known to be responsible for RP development [12].

¹ Metea, M. R., & Newman, E. A. (2007). Signalling within the neurovascular unit in the mammalian retina. *Experimental physiology*, 92(4), 635–640.

² Díaz-Coránguez, M., Ramos, C., & Antonetti, D. A. (2017). The inner blood-retinal barrier: Cellular basis and development. *Vision research*, 139, 123–137.

³ Newman E. A. (2013). Functional hyperemia and mechanisms of neurovascular coupling in the retinal vasculature. *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism*, 33(11), 1685–1695.

⁴ Ivanova, E., Alam, N. M., Prusky, G. T., & Sagdullaev, B. T. (2019). Blood-retina barrier failure and vision loss in neuron-specific degeneration. *JCI insight*, 4(8).

⁵ Sinclair, S. H., & Schwartz, S. S. (2019). Diabetic retinopathy—an underdiagnosed and undertreated inflammatory, neurovascular complication of diabetes. *Frontiers in Endocrinology*, 10, 843.

⁶ Lin, R., Shen, M., Pan, D., Xu, S. Z., Shen, R. J., Shao, Y., ...& Jin, Z. B. (2019). Relationship between cone loss and microvasculature change in retinitis pigmentosa. *Investigative Ophthalmology & Visual Science*, 60(14), 4520–4531.

⁷ Toto, L., Borrelli, E., Mastropasqua, R., Senatore, A., Di Antonio, L., Di Nicola, M., ...& Mastropasqua, L. (2016). Macular features in retinitis pigmentosa: correlations among ganglion cell complex thickness, capillary density, and macular function.



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- Investigative Ophthalmology & Visual Science*, 57(14), 6360-6366.
- ⁸ Kyrkou, A., Stellas, D., Syrrou, M., Klinakis, A., Fotsis, T., & Murphy, C. (2016). Generation of human induced pluripotent stem cells in defined, feeder-free conditions. *Stem Cell Research*, 17(2), 458-460.
- ⁹ Tsohis, K. C., Bagli, E., Kanaki, K., Zografou, S., Carpentier, S., Bei, E. S., Christoforidis, S., Zervakis, M., Murphy, C., Fotsis, T., & Economou, A. (2016). Proteome Changes during Transition from Human Embryonic to Vascular Progenitor Cells. *Journal of Proteome Research*, 15(6), 1995-2007.
- ¹⁰ Markou, M., Kouroupis, D., Badounas, F., Katsouras, A., Kyrkou, A., Fotsis, T., ... & Bagli, E. (2020). Tissue engineering using vascular organoids from human pluripotent stem cell derived mural cell phenotypes. *Frontiers in Bioengineering and Biotechnology*, 8, 278.
- ¹¹ Fligor, C. M., Huang, K. C., Lavekar, S. S., VanderWall, K. B., & Meyer, J. S. (2020). Differentiation of retinal organoids from human pluripotent stem cells. *Methods in Cell Biology*, 159, 279-302.
- ¹² Buskin, A., Zhu, L., Chichagova, V., Basu, B., Mozaffari-Jovin, S., Dolan, D., ... & Lako, M. (2018). Disrupted alternative splicing for genes implicated in splicing and ciliogenesis causes PRPF31 retinitis pigmentosa. *Nature Communications*, 9(1), 1-19.

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The role of ARF6 in human embryonic stem cell pluripotency and differentiation

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ARF6 is a low molecular weight GTPase localized to the plasma membrane and endosomal compartments. As ARF6 cycles through its active (GTP-bound) and inactive (GDP-bound) form, it regulates cell surface ligand internalisation, post internalization trafficking along the endocytic pathway, endosomal recycling, and fusion of recycling vesicles with the plasma membrane. Through its regulator proteins, ARF6 affects many cellular functions including receptor signaling, cell motility, adhesion, abscission, and lipid homeostasis. ARF6 is indispensable during embryonic development, as Arf6 knock-out leads to a lethal phenotype in mice. We are interested in the membrane receptor trafficking and signaling activity of the TGF-superfamily members (TGF, Activin A, and BMP4) in the pluripotency and differentiation of human Embryonic Stem Cells (hESCs). The ActivinA/TGF-family ligands signal via heteromeric complexes of type I and type II transmembrane serine/threonine kinase receptors that phosphorylate the SMAD2/3 proteins to preserve the pluripotent profile of hESCs. The phosphorylated SMAD2/3 proteins oligomerize with SMAD4, translocate to the nucleus, and control transcription via a complex network of interactions with transcription factors, co-activators, and co-repressors. We investigated the function of ARF6 in the phosphorylation of SMADs upon ligand induction using hESCs that overexpress ARF6 or CRISPR-KO lines. We found substantial changes in SMAD phosphorylation in response to ARF6 activation or inactivation, indicating that ARF6 is an important factor in how hESCs react to Activin A TGF family ligands. Here we extend these studies and address the role of ARF6 in differentiation of the above genome edited hESCs to mesendoderm and neuroectoderm. Our results are consistent with an effect of ARF6 in the differentiation to all germ layers. KO ARF6 hESCs exhibit enhanced expression key markers of mesendoderm/ mesoderm (BRACHYURY, MIXL1, WNT3) and extra-embryonic endoderm (AFP2, GATA6 following induction by BMP4. In addition, PAX6, a marker of neuroectoderm differentiation induced under chemically defined conditions was also enhanced in the absence of ARF6. Due to the increased expression of mesodermal markers we further investigated the role of ARF6 in vasculogenesis from mesodermal precursors. We present our findings and discuss their significance.

P57

Thermoresponsive bioink of sodium alginate-based graft copolymers for spontaneous cell-spheroids formation and growth

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We present a self-healable, dually crosslinked, injectable sodium alginate graft copolymer by combining the hydrophobic association of the thermosensitive poly(N-isopropylacrylamide-co-N-tert-butylacrylamide) P(NIPAM_x-co-NtBAM_y) grafted chains and a second ionic association by simple addition of Ca²⁺ cations. The bioink exhibited a two-step gelation mechanism: 1. At room temperature a soft 3D network is formed via the “egg-box” model and 2. Upon thermal triggering of the side chains of P(NIPAM-co-NtBAM) a stronger gel is obtained. The co-existence of the two bonding interactions is crucial for the morphology and the injectability of the gel. Surprisingly, our alginate-based network was found to constitute an excellent cell-spheroid formation matrix by simply mixing the cells with the gel in less than 48h. The viscous gel retains the cells inside its volume favouring the cell-cell adhesion process. The reversible thermo-responsiveness and shear-thinning properties of the gel renders it a promising candidate for cell-spheroids transplantation through injection strategies or the release of the spheroids at room temperature which could find potential applications in disease modeling, drug testing and tissue regeneration.

P58

Brain Milking”: a novel method for the isolation of neural stem cells and oligodendrocyte progenitor cells from live experimental rats

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Postnatal brain neural stem cells (pbNSCs) remain active in specialized niches, where they generate new neurons and glia. One such niche is the subependymal zone (SEZ/ also called the subventricular zone) that is located across the lateral walls of the lateral ventricles adjacent to ependymal cells. Oligodendrocyte progenitor cells (OPCs) are abundantly distributed throughout the central nervous system, constituting a pool of proliferative progenitor cells that can generate oligodendrocytes. Both pbNSCs and OPCs exhibit self-renewal potential and quiescence. Due to their location, the isolation of these cells requires the sacrifice of experimental animals. Here, we describe in detail “brain milking”, a method for the isolation pbNSCs and OPCs from live animals. The protocol has been developed in rats and includes two major steps. First, pbNSCs of the SVZ are “released” from the tissue via the stereotaxic intracerebroventricular injection of a “release cocktail” that contains: a) neuraminidase, a toxin that induces ventricular wall denudation by targeting specifically ependymal cells. b) an integrin-b1-blocking antibody, and c) fibroblast growth factor 2. At a second “collection” step, liquid biopsies of cerebrospinal fluid are performed from the cisterna magna, in anesthetized rats without the need of an incision. Several liquid biopsies can be taken from the same animal on the same and at consecutive days, allowing the performance of longitudinal experiments. By changing the injection co-ordinates, the isolation of different pools of stem/progenitor cells can be achieved.

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P59

Tissue engineering using vascular organoids from human pluripotent stem cell derived endothelial cells and mural cell phenotypes

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Regenerating large tissues requires an intimate supply of the host vascular network, a slow process leading to low viability of the regenerating cells. A solution to this obstacle is the generation of prevascularized tissue engineered constructs. Since Endothelial (ECs) and Mural (MCs) cells, such as smooth muscle cells (SMCs), and pericytes (PCs), are the cellular components of blood vessels and their interactions are crucial for neovascularization, both cell types and their arrangement into correct spatial organization are required in order to rescue tissue engineered constructs from critical ischemia and to form a functional vascular network in vivo. Based on this context and in order to overcome the limitations concerning the isolation and expansion of human SMCs, we developed a protocol to differentiate human pluripotent stem cells (hPSCs) to defined SMC populations (contractile and synthetic hPSC-SMCs) using feeder-free and low serum conditions. hPSC-SMCs phenotypes and hPSC-ECs were extensively characterized concerning their phenotype and function. In addition, we generated ECs from the differentiation of hPSCs (hPSC-ECs). hPSCs-SMCs and ECs (hPSC-ECs or primary ECs), using a methylcellulose-based hydrogel system, were then used to generate 3D vascular organoids, which rapidly give rise to a complex three-dimensional vascular network. Vascular organoids (ECs and hPSC-SMCs) were extensively characterized regarding their phenotype, cell-cell interactions and their ability to form a three-dimensional capillary network in vitro. Finally, we investigated the vascularization potential of these vascular organoids, when embedded in hydrogels composed of defined extracellular components (collagen/fibrinogen/fibronectin) that can be used as scaffolds in tissue engineering applications. To sum up, we developed a robust method for the generation of defined hSMCs phenotypes from hPSCs. In addition, we differentiated hPSCs to ECs. Fabrication of hECs/hPSC-SMC vascular organoids embedded in chemically defined matrices is a significant step forward in tissue engineering and regenerative medicine.

**This research is co-financed by Greece and the European Union (European Social Fund- ESF) through the Operational Program «Human Resources Development, Education and Lifelong Learning 2014- 2020» in the context of the project «Generation of distinct phenotypes of mural cells from differentiation of human pluripotent stem cells: application in the generation of vascularized tissue engineered constructs» (MIS 5047550).*

P60

Effect of grape stems extract on viability of Subependymal Zone derived neural stems cells, on behavior and on acetylcholinesterase activity in brain regions of adult male mice

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The winemaking procedure results in the production of stems, by-products that are not environmentally friendly. However, grape stems are rich in polyphenols and, therefore, they are putatively beneficial for human health. Simultaneously, grape stems are rich in polyphenols and, therefore, they are putatively beneficial for human health. The aim of the present study was to investigate the effect of the grape stems extract a) on viability of Subependymal zone (SEZ) derived Neural Stems Cells (NSCs) b) on anxiety-like behavior and c) on the activity of two isoforms of acetylcholinesterase in specific brain regions (cortex, striatum, hippocampus, cerebellum, midbrain) of adult male mice. The grape stem extract (GS extract) was derived from a native Greek vine, namely Mavrodaphne and was rich in polyphenols (205.2 mg/g extract). NSCs were treated with 4 dilutions (20, 10, 5, 2.5 µg/ml) of GS extract on proliferation and differentiation conditions. Cell viability was determined by the MTT chromogenic assay 24h after the treatment. Furthermore, the GS extract was administrated orally (gavage, 155.9 mg/Kg body weight) to adult C57BL/6 male mice for 28 days (long-term). Anxiety-like behavior was assessed by using the open-field test, followed by video-tracking software (Any-maze 6.3). Acetylcholinesterase activity was determined in both salt-soluble and detergent-soluble fractions of specific brain regions (cortex, striatum, hippocampus, cerebellum, midbrain) of male mice, by using Ellman's colorimetric method. Results suggested that the GS extract administration on NSCs had no effect on cell survival, although a tendency in increased proliferation was noted at the concentration of 5 µg/ml treatment. Anxiolytic-like behavior was observed after the stem extract administration. Stem extract treatment reduced acetylcholinesterase activity of both isoforms in various brain regions. In conclusion, long term administration of grape stem extract, rich in polyphenols, appears to have anxiolytic as well as acetylcholinesterase inhibitory activity.

P61

Metabolomics as quality control tool in precision manufacturing of microengineered complex joint implants

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Osteoarthritis (OA) is the most prevalent arthritic disease affecting 25% of the adult population. OA involves the entire joint affecting both the articular cartilage and the underlying bone. Hence, it is crucial to consider the entire osteochondral unit as a target for repair. Engineered joint implants could provide a solution for this type of defects, preventing OA progression. However, currently the relevant tissue-engineering manufacturing processes have not been standardized, lacking the consistency required for their regular application in clinical practice. Quality control protocols and appropriately tailored automated manufacturing procedures need to be set up to increase their reproducibility and minimize any variations in the quality of the final product and the success of the tissue regeneration. The EC Horizon 2020 project JOINTPROMISE aims towards the development of such automated platform with capability to produce large, patterned and vascularised joint implants. JOINTPROMISE paves the way for high-volume, affordable production of entire biological joints, addressing a major socioeconomic challenge of the European ageing society.

Metabolomics has been proven as a sensitive monitoring tool of bioprocess consistency, providing a holistic perspective of the cellular metabolic physiology. In the case of engineered joint implants, endometabolomics of micro- and selected macro-tissues will provide insight on the effects that bioprinting could have on the final result, leading to appropriate adaptations of the process parameters to minimize impact. The available knowledge and data are currently limited. Correlation of endo- with exo-metabolomic data are to provide non-destructive quality control markers, which could alarm about any changes at various steps of the project. In this study, we will demonstrate all stages of the process and indicate where samples for metabolic profiling are to be acquired. The obtained data will contribute to the resources used for the development of implementation rules for these manufacturing processes from the official regulatory bodies.

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P62

Exploring the contribution of PML in neuronal cell specification and survival

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The Promyelocytic Leukemia Protein (PML) originally characterized as a tumor suppressor is the core organizer of cognate nuclear bodies (PML-NBs) that regulate various physiological processes in different cell types. In embryonic (ESCs) and induced pluripotent (iPSCs) stem cells we have recently reported that PML is required for maintenance of the naïve and acquisition of the induced pluripotency state, respectively. The role of PML in the nervous system is poorly exploited. In this work we examine the role of PML in neuroprotection and neurodegeneration. We show that neuroectodermal differentiation of ESC is strongly reduced following ablation of PML. Employing eNSC (E13.5) from WT and PML ^{-/-} mice, we found that PML influences the differentiation pathway choice favoring the neuronal and inhibiting the astrocytic lineage. The survival of PML KO eNSCs following oxidative, genotoxic or beta-amyloid stress was impaired in comparison to the WT. PML KO eNSCs present lower mitochondrial potential and higher ROS levels compared with the WT, reinforcing the hypothesis that key mitochondrial mechanisms are disrupted when PML is absent. Furthermore, the ablation of PML causes a reduction in the autophagic flux as measured by immunodetection of LC3-II. The activation levels of Akt, GSK3 β , mTOR and p-70S6 are reduced in the absence of PML, indicating that it is acting via the PI3K/AKT/mTOR axis, a signaling pathway elicited by neurotrophic factors. In conclusion, PML is required for the neuronal specification of eNSC and their response to oxidative, genotoxic and beta-amyloid stress. In addition, it maintains the autophagy flux and the mitochondrial integrity. We are currently exploiting the involvement of PML in neurodegeneration using cell and animal models for Alzheimer's Disease.

P63

The wild and the tamed; a comparison of the activity of postnatal brain neural stem and progenitor cells between lab mice and the fossorial species *Microtus thomasi*

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Postnatal brain neural stem and progenitor cells (NSPCs) cluster within stem cell niches contributing to odour recognition, learning, memory and myelination, with their activity being modified by exercise, stress and environmental enrichment. Here, we investigate the hypothesis that lab-mouse NSPCs might remain in a “tamed”, hypoactive, status, due to their maintenance in very controlled conditions. We use lab mice and *Microtus thomasi* animals, the latter being a fossorial species. We analyze brain samples, by immunohistochemical staining for markers specific for cell proliferation (PCNA, Ki67) and neural progenitor identity (Sox2, Dcx and GFAP), focusing on the Subependymal Zone and the dentate gyrus stem cell niches, as well as on the olfactory bulbs. Furthermore, we culture NSPCs from the same animals and we assess similar markers immediately after isolation and after two or three passages. Our data reveal increased density and mitotic activity of NSPCs in the Subependymal Zone of wild species, with no changes in the hippocampus and the corpus callosum. We have also confirmed the ability to culture NSPCs from wild animals and we describe differences both in terms of morphology and behavior. Our data indicate that the activity of pools of postnatal brain NSPCs are affected by external “life-style” stimuli. Whether such differences are hard-wired in NSPCs or are continuously maintained remains open, but can be addressed by this type of analysis. The above are important in terms of assessing the limitations of using lab animals in experimental analyses.

P64**The role of placental CRH in human brain development**

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Among mammals, anthropoid primates display a unique expression pattern of feto-maternal hormonal interaction. During pregnancy, their placenta is progressively producing enormous amounts of the Corticotropin-Releasing Hormone (CRH). Although the role of hypothalamic CRH has been extensively studied in the physiology of stress, there is a remarkable lack of evidence regarding the role of placental CRH, while the biological significance of its unique expression pattern in anthropoid primate species remains elusive.

In order to investigate the effects of placental CRH on human brain development and to overcome the limitations raised in experimenting with human tissue, we have generated human 3D-neural spheroids and human cerebral cortical organoids from human embryonic stem cells (hESCs). Exposure of neural spheroids to CRH during a 50 days culture period, results in significant differences in the size and the cellular composition of the spheroids as assessed by immunohistochemical detection of specific markers of the neural lineage. Pharmacological disruption of the CRH signaling reverses the effects of CRH in the same culture model. We also revealed altered cellular fate, gene expression and architectural characteristics in 50 days human cerebral cortical organoids.

The key role of CRH in stress physiology and the human-specific pattern of placental CRH expression, suggest that this in vitro approach provides a unique tool for our understanding of the mechanisms underlying the role of stress hormones in human physiology and raises the intriguing possibility for pharmacological applications in neurodevelopmental disorders and deficits associated with anxiety.

P65

Decreased differentiation capacity and altered expression of extracellular matrix components in irradiation-mediated senescent human breast adipose-derived stem cells

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Radiotherapy is widely used for the treatment of breast cancer. However, we have shown that ionizing radiation can provoke premature senescence in breast stromal cells. In particular, breast stromal fibroblasts can become senescent after irradiation both in vitro and in vivo and they express an inflammatory phenotype and an altered profile of extracellular matrix components, thus facilitating tumor progression. Adipose-derived stem cells (ASCs) represent another major component of the breast tissue stroma. They are multipotent cells and due to their ability to differentiate in multiple cell lineages they play an important role in tissue maintenance and repair in normal and pathologic conditions. Here, we investigated the characteristics of human breast ASCs that became senescent prematurely after their exposure to ionizing radiation. We found decreased expression levels of the specific mesenchymal cell surface markers CD105, CD73, CD44 and CD90. In parallel, we demonstrated a significantly reduced expression of transcription factors regulating osteogenic (i.e. RUNX2), adipogenic (i.e. PPAR γ) and chondrogenic (i.e. SOX9) differentiation; this was followed by an analogous reduction in their differentiation capacity. Furthermore, they overexpress inflammatory markers, i.e. IL-6, IL-8 and ICAM-1, and a catabolic phenotype, marked by the reduction of collagen type I and the increase of MMP-1 and MMP-13 expression. Finally, we detected changes in proteoglycan expression, e.g. the up-regulation of syndecan 1 and syndecan 4 and the down-regulation of decorin. Notably, all these alterations, when observed in the breast stroma, represent poor prognostic factors for tumor development. In conclusion, we showed that ionizing radiation-mediated prematurely senescent human breast ASCs have a decreased differentiation potential and express specific changes adding to the formation of a permissive environment for tumor growth.

Reference

Papadopoulou A. et al., *IUBMB Life*. 2022 Oct;74(10):969-981.

P66

A subset of human skin fibroblasts exposed to UVB radiation can escape premature senescence

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Normal cells when exposed to exogenous genotoxic stresses can become prematurely senescent. These cells express a specific senescence-associated secretory phenotype (SASP), which affects tissue homeostasis, including promotion of tumor growth. On the hand, UV radiation accelerates the ageing of the skin, a phenomenon called "photoageing". In this line, it has been previously shown that the high-energetic and genotoxic UVB radiation is able to provoke, in a short period, premature senescence in human skin fibroblasts (HSF). Here, we exposed HSF repeatedly to non-cytotoxic doses of UVB (10 doses of 35 mJ/cm²) and found that after a few days the cells present signs of premature senescence, i.e. inhibition of proliferation and expression of senescence-related markers. However, we observed in a long-term culture that irradiated cells form a mixed population composed by senescent and proliferating cells; this is in contrast to ionizing radiation-treated cells which form a population of solely senescent cells. These, proliferating, so-called "escape", cells remain normal as they have a limited life-span and they respond normally to stresses, such as an oxidative stress or an additional exposure to UVB, similarly to unexposed HSF. RNAseq analysis showed that "escaped" cells constitute an intermediate population between young and ionizing radiation-induced senescent cells. This has been verified by expression analysis with qPCR. Among the genes that have been tested there are several classical SASP genes, such as those coding for inflammatory cytokines and matrix metalloproteases, among others. Interestingly, these components are known from our previous work to enhance the growth of cancer cells in vitro and in vivo. In agreement, "escaped" cells enhance the growth of colonies of A431 squamous cancer cells in co-cultures with fibroblasts. However, the role of these cells on tumor growth and on tissue homeostasis, in general, must be tested in appropriate models in vivo.

P67

Anti-Aging”: Exploitation of Greek microbial diversity for the development of innovative cosmeceuticals and food supplements

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Greek ecosystems are a fertile yet under-explored ground for the study of Actinobacteria-known producers of bioactive compounds. By exploring this under-investigated biodiversity and chemodiversity of Greek actinobacterial strains (mostly of the genus *Streptomyces*), the “AntiAging” project aims to uncover potentially novel natural compounds with anti-aging activity that can be formulated as cosmeceutical and nutraceutical products. In total, 3000 isolates belonging to the Athens University Bacterial & Archaea Culture Collection (ATHUBA)-some originating from unique environments (caverns, volcanoes, thermal springs, etc.)-were studied. A customized *in-house* library of 2000 extracts was generated, all of which were investigated for potential anti-aging properties. This biological evaluation was performed through elastase (anti-aging activity) and tyrosinase (whitening activity) inhibition *in vitro* assays. Fractions (~400) of the most promising, highly active compounds are chosen for the same biological evaluation and assessment of their possible cell toxicity (~400). Next, metabolites (~30) of the most nontoxic extracts and fractions, are biologically evaluated for their anti-aging properties, whitening activities, antioxidant potential, proteasome and autophagy-lysosome system activation capability. Last, the most promising metabolites are tested in a *Drosophila melanogaster* *in vivo* model, where we evaluate their ability to induce antioxidant mechanism through activation of the Nrf2/cncC transcriptional factor. A small yet significant number of extracts demonstrated >50% elastase inhibitory activity, whereas a considerably larger number exhibited >50% tyrosinase inhibitory action. Interestingly, in some cases, the bioactivity exceeded 80% in *in vitro* assays. The latter results show a hidden yet powerful potential of harnessing Greek microbial wealth in the context of the science of anti-aging.

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P68

The disruption of ubiquitin homeostasis, following *Usp14* KD, unveils the functional wiring of proteostatic modules with major bioenergetic signaling cascades and DNA damage response mechanisms

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Age-associated phenotypes are mainly caused by the progressive accumulation of biomolecular damage that along with the gradual declining capacity of cellular stress response mechanisms lead to the deterioration of tissue homeostasis. Since cells are mainly composed from highly sophisticated protein machines, organisms have developed protein quality control systems, such as the proteostasis network (PN), to limit loss-of-proteostasis. Deubiquitinase enzymes (DUBs) are major components of proteostatic modules that govern selective spatio-temporal protein turnover; DUBs reverse ubiquitin signals and, thereby, modulate (among others) ubiquitin homeostasis. *Usp14* (one of the three deubiquitinases) by directly binding to proteasome, directs protein degradation at three distinct levels, i.e., a. the recognition and binding of the ubiquitinated substrate, b. the translocation of the substrate to the inner proteasome chamber and c. the recycling of polyubiquitin chains. Here, we aimed to characterize of physiological alterations following *Usp14* knock down (KD) in *Drosophila melanogaster*. Our data suggest that *Usp14* KD-mediated disruption of ubiquitin homeostasis, results in dose- and tissue-dependent proteostatic, mitostatic and metabolic cell/tissue adaptations. Interestingly, suppression of protein deubiquitination, also triggered the accumulation of DNA damage and, in turn, activated DNA damage response mechanisms. Our findings highlight the pivotal role of *Usp14* in the modulation of proteome and genome stability.

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Biological evaluation of extracts obtained from Greek flora plants exerting anti-aging and skin-protective properties

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Natural products are enriched repositories for the identification of novel bioactive compounds with cosmeceutical, pharmacological or even disease treating properties. Greece is known for its remarkable biodiversity, so there is an increasing interest in studying the Greek flora. During the *CosmAGE* project we are performing an extensive high-throughput screening of natural products isolated from various plants of the Greek flora, aiming to identify novel bioactive molecules with potential anti-aging and/or cosmeceutical properties. Plants from different areas were collected and extracted using two different techniques, the Supercritical Fluid Extraction (SFE) and the Accelerated Solvent Extraction (ASE). The extracts obtained were examined in normal human cells for their ability to activate modules of the proteostasis network, for suppressing oxidative stress and also for their potential skin protective effects as evidenced by effective inhibition of the skin aging-related enzymes, elastase and tyrosinase. Our preliminary findings have revealed several promising extracts that could be a potential source of novel bioactive molecules with anti-aging properties.

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Biological evaluation of edible green plants growing in Greece

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The Mediterranean diet is renowned for its beneficial properties on human health, since it has been proven to contribute to decreased rates of heart disease, cancer, and neurodegenerative diseases. Therefore, it provides an excellent repository for the discovery of new bioactive natural products. Leafy green vegetables are an integral part of the Greek dietary regime. Herein, samples of plant species belonging to the family Compositae were studied for their content in bioactive compounds. Specifically, *Centaurea raphanina*, *Sonchus tenerrimus* and *Sonchus asper* were collected and extracted using different techniques. The extracts obtained were evaluated for their possible activation of proteostasis network components and antioxidant activity by performing cell-based assays. All examined extracts showed no significant toxicity to BJ normal human skin fibroblasts and HaCaT immortalized human keratinocytes. Interestingly, the extracts of *C. raphanina* were found to reduce the intracellular oxidative load and to also induce the expression of antioxidant responses-related genes in BJ fibroblasts or HaCaT keratinocytes; further the extracts of *S. asper* and *S. tenerrimus* exerted significant antioxidant activity in human keratinocytes. These findings indicate that the studied plants are likely promising sources for the isolation of novel bioactive protective and/or therapeutic agents.

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Role of *Pgc-1 α /srl* in proteostasis network regulation and mitochondrial biogenesis

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Mitostasis maintains mitochondrial number and quality since mitochondrial mass and activity must be adjusted in response to the regulation of tissue homeostasis, cell growth rates, and nutrient availability. In mammals, the transcriptional coactivator family PGCs (PPAR- γ coactivators) regulate multiple metabolic processes, including mitochondrial biogenesis. Being dynamic cellular structures, mitochondria also rapidly remodel their shape and size through fusion and fission. On the other hand, the proteostasis network (PN) ensures protein quality control during normal conditions and under increased proteotoxic stress. Although mitostasis appears to be regulated by the PN, the response of the main proteolytic mechanisms following mitostasis disruption remains largely unknown. We show here in the fly model the involvement of the transcriptional co-activator *srl/Pgc-1 α* in the regulation of the PN. Although *srl/Pgc-1 α* interacts with *Nrf2/cncC* (a master regulator of PN) to induce mitochondrial biogenesis, *srl/Pgc-1 α* -mediated activation of the PN is not *cncC/Nrf2*-dependent; notably, *cncC/Nrf2* appears to be crucial for the regulation of mitochondrial gene expression, metabolism, mitochondrial respiration, and oxidative load. *srl/Pgc-1 α* was found to act complementary to *cncC/Nrf2* to compensate for its genetic deficiency in maintaining mitostasis and improving flies' longevity. Finally, we show that suppression of the inner mitochondrial membrane fusion activates *srl/Pgc-1 α* to induce UPP upregulation, improvement of mitochondrial homeostasis, and increased longevity. Thus, activation of proteostatic mechanisms during impaired mitostasis appears to be regulated by the action of both the transcription factor *cncC/Nrf2* and the transcriptional cofactor *srl/Pgc-1 α* . Our findings indicate that the toxicity induced by mitochondrial dysfunction can be partially rescued through the activation of the *srl/Pgc-1 α* transcription cofactor.

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POSTER SESSION 2

FUNCTIONAL GENOMICS & PROTEOMICS -

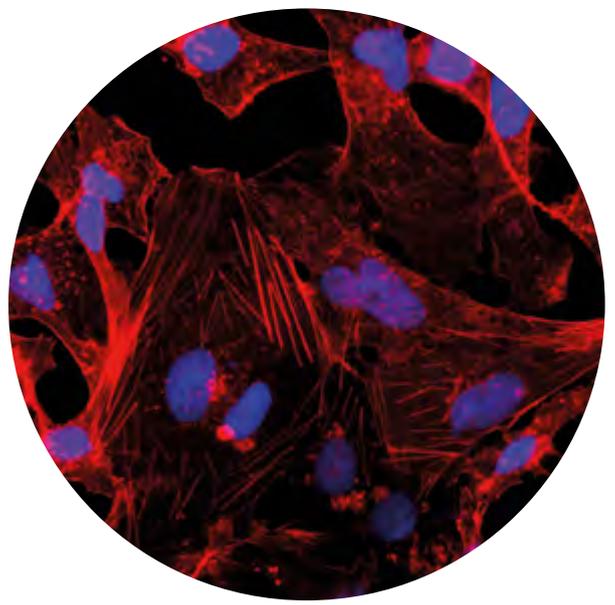
DNA DAMAGE/REPAIR

CELL COMMUNICATION & SIGNALING -

DEVELOPMENT & DIFFERENTIATION

CELL ORGANIZATION & FUNCTION -

BIOTECHNOLOGY OF PLANTS & MICROORGANISMS



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Bioactivity in fermented foods: an *in silico* study of the peptides of yogurt and wine

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In recent years, bioactive peptides present in foods and their potential beneficial effects for human health have been under rigorous investigation. Bioactive peptides are released during the fermentation process from the proteins of raw materials (e.g. milk and must) as a result of the proteolytic activity of the fermenting microbiome which is mainly comprised of lactic acid bacteria (LAB) and yeasts. The aim of our study was to collect the publicly-available peptidomics datasets from different wines and yogurt samples and re-evaluate *in silico* their physicochemical properties and functionalities using state-of-the-art bioinformatic tools. The majority of the peptides found in yogurt derived from the hydrolysis of β -casein, while others from α_{s1} -, α_{s2} -, and κ -casein. Very few peptides derived from the hydrolysis of whey proteins (β -LG and α -LA). Peptides in wine mainly come from the partial conversion of either naturally occurring proteins (i.e., the proteomes of *Vitis vinifera* and *Saccharomyces cerevisiae*) or from exogenous proteins added for technological reasons. For the physicochemical properties of the peptides, various parameters were calculated, including aliphatic index, and grand average of hydropathicity (GRAVY), using software tools such as ProtParam. For the functional characterization of the peptides, we used the following databases: FermFoodDB, DFBP, BioPepDB, BioPep-UWM and MBPDB. Some of the aforementioned databases, such as EROP-Moscow allowed us to correlate the peptides with the flavor of foods (sour, umami or bitter). The bioactive peptides were found to exhibit diverse activities, such as antioxidant, antihypertensive, antimicrobial, anti-inflammatory, immunomodulatory and opioid. Interestingly, some reported peptides present more than one functions. More studies need to be performed to further validate the bioactivity of these peptides so that they find application in the food industry.

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Drug repurposing in CLL based on selected high quality proteomic data

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Chronic Lymphocytic Leukemia (CLL) is a hematological malignancy that exhibits high molecular heterogeneity and limited effectiveness in disease treatment. One big challenge for this disease, is to investigate the molecular characteristics of each patient in order to deliver more personalized therapeutic regimes. Proteomic analysis represents the best approach to unravel such proteome heterogeneity and highlight significant pathways that drive tumorigenesis. Proteomics can, also, be exploited to repurpose already known and safe FDA-approved drugs, thus minimizing the time and cost required for de novo drug development.

In our recent publication¹, we collected all available proteomic and drug repurposing studies in CLL, and proposed novel methods to utilize this knowledge in drug identification. In relation to this concept, in the present work we integrated and reanalyzed (comparative analysis, pathway enrichment analysis, functional characterization, clustering, network analysis, drug repurposing and drug evaluation) selected high quality proteomic data with state-of-the-art bioinformatic tools. Specifically, we integrated proteomic data from 35 patients and 12 healthy individuals, which represents the largest analysis of healthy vs CLL samples dataset up to date. By collectively analyzing this data we found that approximately 1000 proteins create a unique proteomic landscape in CLL and distinguish cancer cells from healthy ones, thus driving tumorigenesis. These proteins work together to create complex networks and affect major pathways and processes within the cell, such as metabolism, stress response and RNA processing. Additionally, we bioinformatically matched altered proteins with drugs to re-identify various known drugs and, more significantly, propose new chemical compounds that may combat CLL. In conclusion, our method leverages existing proteomic data from various sources and moves one step further towards the identification of selective compounds against hematologic malignancies.

Bibliography

¹ Mavridou Dimitra, Konstantina Psatha, and Michalis Aivaliotis. 2021. "Proteomics and Drug Repurposing in CLL towards Precision Medicine" *Cancers* 13, no. 14: 3391. <https://doi.org/10.3390/cancers13143391>

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Transcriptomic analysis in modelled osteoporosis reveals coding and non-coding RNAs as new potent regulators of bone remodeling

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Osteoporosis is a multifactorial disease characterized by bone loss, bone fragility and increased bone fracture risk and is often undiagnosed until the appearance of bone fractures. Our lab has established a genetic mouse model of osteoporosis by overexpression of human RANKL in transgenic mice (TgRANKL). In the current study, we identified differentially expressed (DE) genes with potential clinical value in osteoporosis by performing RNA-Seq for mRNAs, miRNAs, and lncRNAs in flushed femurs from TgRANKL and control wild-type (WT) mice. Selected genes were validated with qPCR in femurs from WT mice, untreated TgRANKL mice and treated with an anti-RANKL therapy (Denosumab). Regarding mRNAs, we identified in total 2,747 DE mRNAs ($|\log_2\text{FoldChange}| > 1$, adjusted p-value < 0.05), 959 of them being upregulated and 1,788 downregulated in TgRANKL femurs compared to WT. Enrichment analysis of the upregulated genes revealed that they were related to protein degradation, peptidase activity, and bone remodelling, while downregulated genes were related to oxidative phosphorylation, and metabolism. The expression of 39 confirmed upregulated genes returned to normal levels after Denosumab treatment, while it was examined in osteoclastogenesis and osteoblastogenesis experiments, too. Concerning miRNAs, we identified 63 DE miRNAs ($|\log_2\text{FoldChange}| > 1$, adjusted p-value < 0.05), 33 of them being upregulated and 30 downregulated. We validated with qPCR, 4 upregulated and 5 downregulated miRNAs. Regarding lncRNAs, we identified through RNA-Seq 235 DE lncRNAs ($|\log_2\text{FoldChange}| > 2$, adjusted p-value < 0.05), 80 of them being upregulated and 155 downregulated. The DE genes revealed in this study may serve as the basis for the discovery of novel pathogenic mechanisms and the identification of new biomarkers in osteoporosis or as potential drug-targets.

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Pulmonary infection by SARS-CoV-2 induces senescence accompanied by an inflammatory phenotype in severe COVID-19: possible implications for viral mutagenesis

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SARS-CoV-2 infection of the respiratory system can progress to a multi-systemic disease with aberrant inflammatory response. Cellular senescence promotes chronic inflammation via senescence-associated secretory phenotype (SASP). We investigated whether COVID-19 disease is associated with cellular senescence and SASP. Autopsy lung tissue samples from 11 COVID-19 patients and 43 age-matched non-COVID controls with similar comorbidities were analysed by immunohistochemistry for SARS-CoV-2, markers of senescence and key SASP cytokines. Virally-induced senescence was functionally recapitulated *in vitro*, by infecting epithelial Vero-E6 cell line and a 3D alveosphere system of alveolar type 2 (AT2) cells with SARS-CoV-2 strains isolated from COVID-19 patients. SARS-CoV-2 was detected by immunocytochemistry and electron microscopy predominantly in AT2 cells. Infected AT2 cells expressed the angiotensin-converting-enzyme 2 (ACE2) and exhibited increased senescence (p¹⁶INK4A and GL13 positivity) and IL-1 β and IL-6 expression. *In vitro*, infection of Vero-E6 cells with SARS-CoV-2 induced senescence (GL13), DNA damage (γ -H2AX), increased cytokines (IL-1 β , IL-6, CXCL8) and



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Apolipoprotein B mRNA-editing (APOBEC) enzyme expression. Next-generation-sequencing analysis of progenies obtained from infected/senescent Vero-E6 cells demonstrated APOBEC-mediated SARS-CoV-2 mutations. Dissemination of the SARS-CoV-2-infection and senescence was observed in extra-pulmonary sites (kidney and liver) of a COVID-19 patient. We demonstrate that in severe COVID-19, AT2 cells infected by SARS-CoV-2 exhibit senescence and a proinflammatory phenotype. *In vitro*, SARS-CoV-2 infection induces senescence and inflammation. Importantly, infected senescent cells may act as a source of SARS-CoV-2 mutagenesis mediated by APOBEC enzymes. Therefore, SARS-CoV-2-induced senescence may be an important molecular mechanism of severe COVID-19, disease persistence and mutagenesis

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Tumor mutational burden assessment in non-small-cell lung cancer samples and correlation with the tumor microenvironment

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Lung cancer is among the most frequent malignancies worldwide, characterized by high mortality rate and is strongly related to tobacco smoking. The majority of reported cases concerns non-small cell lung cancer (NSCLC), being further subdivided in distinct subtypes. The second most common is squamous cell carcinoma (SCC) that accounts for 25-30% of all NSCLC cases. Although the outcome of NSCLC patients has considerably improved the past few years owing to novel targeted and personalized therapeutic approaches, mainly of immune checkpoint inhibitors targeting programmed cell death receptor-1 (PD-1) or its ligand (PD-L1), the overall survival rates still remain low. Tumor microenvironment and its dynamic and complex interaction with tumor cells are actively implicated in prognosis and response to therapy. Tumor infiltrating lymphocytes, in particular, have been acknowledged as a crucial component that affects tumor growth and clinical outcome. Lung cancer is a molecularly heterogeneous disease with a differential cohort of gene mutations in each subtype. Next generation sequencing (NGS) has become an indispensable tool in assessing the mutational and genomic profile of each patient in order to acquire the optimal treatment. New targeted sequencing panels are sufficiently effective to estimate tumor mutation burden (TMB) within a tumor genome, referring to the number of somatic mutations per megabase of exonic sequence harbored by tumor cells. In this study, assessment of TMB was performed by targeted next-generation sequencing in NSCLC. TMB was measured by OncoPrint™ Tumor Mutation Load in 24 retrospective SCC patients. Archival FFPE tissue samples were selected based on peritumoral lymphocytic reaction and classified in two categories, low and high grade, accordingly. Tumor size, cellularity and minimal necrosis were also taken into consideration. Data regarding the assessment of TMB in the 24 SCC samples and its potential correlation with the tumor microenvironment, especially tumor infiltrating lymphocytes, will be presented and discussed.

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Proteomic analysis of white muscle tissue of *Engraulis encrasicolus* (European anchovy) - Initial results

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Background/Aim: *Engraulis encrasicolus* is a small pelagic species, commonly known as European anchovy, which in Greece is mainly fished in the North Aegean Sea and the Thermaikos Gulf. Anchovy fisheries make up 1/10 of the total annual catch of the greek fishing fleet, therefore fishing and trading of this species plays an important role in the local and national economy. Moreover, anchovy is of high nutritional value for human consumption and an integral part of the mediterranean diet. In this prospect, studies investigating the preservation of its nutritional value, either as a fresh or processed product, are of both scientific and commercial interest. Within this context, we embarked on the characterization of the proteome of the white muscle of anchovy, as this corresponds to the major portion of the edible part of the fish.

Materials and Methods: White muscle tissue samples excised from n=3 fish were investigated. Total proteins were extracted from tissues processed by the FASP method and analyzed using nano-liquid chromatography coupled to an Orbitrap mass spectrometer (nanoLC-ESI-Orbitrap MS/MS). MS data underwent bioinformatics processing using known sequences from other fish species available on the SWISS-PROT/UniProt database, since there exist no data on the European anchovy proteins.

Results: Our analyses resulted in identification of 386 proteins, of which one belonged to the parasitic worm *Anisakis pegreffii*. Protein stratification according to biological process, showed that 30.5% of proteins were of unknown function, 11.1% were proteins involved in metabolic processes, 9.9% were signaling proteins, 9.3% were transporters, 9.1% were involved in protein modification, 3.9% were cytoskeletal proteins and the rest 24.3% had other functions.

Conclusion: The present study represents the first attempt to characterize the proteome of European anchovy. Our results contribute to the enrichment of the corresponding databases. Moreover, they form the basis for the identification of protein-markers of the species' spoilage process.

Ευχαριστίες: Η παρούσα εργασία υλοποιήθηκε στο πλαίσιο του έργου «Επέκταση εμπορικής διάρκειας ζωής του νωπού γαύρου σε πάγο με χρήση μικρο-νανο-φυσαλίδων όζοντος» (ΟΠΣ/ΜΙΣ 5010351), που χρηματοδοτήθηκε από το Ε.Π.ΑΛ.Θ. (ΕΣΠΑ 2014-2020). Ευχαριστούμε επίσης το πλήρωμα του αλιευτικού σκάφους «Σ. ΜΑΝΙΟΣ» και ιδιαίτερα τον ιδιοκτήτη και καπετάνιο του κ. Ι. Μανιό.

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Early response to hypoxia involves significant alterations in the methyl-arginine proteome

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Cell adaptation to hypoxia entails transcriptional reprogramming, which is mainly mediated by Hypoxia Inducible Factors. However, various transcription-independent processes also occur at the onset of hypoxia as an early response to the reduction of oxygen levels. These include alterations in cellular architecture, mRNA processing and epigenetic marking of chromatin. The latter usually involves oxygen sensitive enzymes such as the JmjC family of N-methyl lysine demethylases (KDMs) previously shown to facilitate the response to hypoxia¹. However, in addition to targeting lysine residues inside histone molecules, methylation can also modify arginine residues usually found in arginine- and glycine-rich motifs (RGG/RG) participating in nucleic acid binding and protein-protein interactions². Here, using a methyl-arginine specific antibody to immunoprecipitate and enrich for modified proteins, we show that brief exposure (2 h) of MCF7 cells to hypoxia significantly altered the methyl-arginine landscape of proteins. Proteomic analysis revealed that most of the identified proteins (104 out of 142 proteins). exhibited decreased arginine methylation under hypoxia. These proteins are mainly involved in RNA processing, regulation of gene expression (including histone variants) and intracellular trafficking. On the other hand, a smaller, but equally significant, subset of proteins (38 out of 142), involved in processes such as cytoskeleton organization, exocytosis, and cell cycle regulation, exhibited increased levels of arginine methylation in the hypoxic cells. So far, our data suggest that the cellular response to hypoxia entails a fast alteration in the arginine methylation profile of proteins that may be important not only for reprogramming gene expression but also for cell architecture and cell cycle control that are also affected by hypoxia.

¹ Batie et al. (2019) *Science* 363: 1222-1226.

² Blanc et al. (2017) *Mol. Cell* 65: 8-24.

P80

Proteomic profiling of CYLD-deficient mammary epithelial cells for the identification of signaling molecules and pathways that mediate EMT

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CYLD is a deubiquitinating enzyme that preferentially hydrolyzes K63- and M1-linked polyubiquitin chains and thus regulates the assembly of critical signaling complexes that form on polyubiquitin chains in response to receptor activation. It is often downregulated or mutated in human cancers. We have recently shown that CYLD downregulation or inactivation induces epithelial to mesenchymal transition (EMT) in human mammary epithelial cells, which is considered a critical process for the development of metastatic phenotype of various tumors. Our hypothesis is that CYLD regulates EMT by deubiquitinating and modulating the function of specific molecules that in turn regulate specific signaling pathways, such as the TGF- β and Hippo pathways. To elucidate that mechanism, we employed a comparative mass-spectroscopic (MS) analysis of whole cell lysates from control and CYLD-deficient MCF10A clones. Our analyses revealed over 3.000 statistically significant differentially expressed proteins and phosphorylated peptides in CYLD-deficient MCF10A clones. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses indicated significant differences related to endoplasmatic reticulum (ER) stress and the unfolding protein response (UPR) pathway. These findings suggest an involvement of CYLD in integrated stress response (ISR) which may underlie EMT. We are currently investigating the functional and biochemical relationship of CYLD to specific mediators of ISR and its role in the survival and fitness of CYLD-deficient mammary epithelial cells.

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A versatile multiplex nanopore sequencing approach reveals the transcriptional profile of *MAPK1* in multiple human malignancies

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The superfamily of eukaryotic protein kinases (ePKs) comprises the extensively studied mitogen-activated protein kinases (MAPKs) family, a subgroup of cellular regulators that is involved in a series of pivotal signal transduction pathways. Previous studies have already shown that alternative splicing events in most members of the MAPKs can determine the desired cell fate in stress responses. Although the involvement of the already identified alternative MAPK transcript variants in multiple signaling cascades has been clarified, the transcriptional landscape of the vital *MAPK1*, has not been investigated. In the present study, we developed and implemented a multiplexed nanopore sequencing methodology to examine the potential existence of novel *MAPK1* splice variants in a wide panel of human cell lines. The implemented cutting-edge barcoded nanopore sequencing approach enabled the identification of novel full-length *MAPK1* mRNAs and elucidated their expression pattern in various human malignant tissues as well as in non-cancerous cell lines. In the framework of the current study, 10 previously undescribed *MAPK1* mRNAs (*MAPK1* v.3 – v.12) were detected, while the multiplexing options of nanopore technology enabled the evaluation of their relative expression levels. Furthermore, the optimization and employment of qPCR assays validated each newly identified *MAPK1* mRNA in a wide spectrum of human cell lines with notable specificity. Finally, *in silico* analysis unveiled the protein-coding capacities for 4 novel *MAPK1* mRNAs that harbor open reading frames, hence are highly expected to be implicated in the regulation of MAPK pathways, demonstrating differential localizations and functionalities. Undoubtedly, further research is an imperative need for the elucidation of the complex role of *MAPK1* under normal and/or pathological conditions in order to clarify the potential characterization of the novel mRNAs as prognostic and/or diagnostic biomarkers or therapeutic targets.

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Exploring the role of 3D cell microenvironment through proteogenomics profiling for drug repurposing

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Introduction

Drug repurposing a. reveals new molecular targets, b. expands pharmaceutical research and c. eases the efficacy/toxicity burden. Herein, we exploit the 3D cell microenvironment to identify druggable key-players and candidate companion biomarkers. For this, LC-MS/MS and high-resolution particle-by-particle 3D fluorescent microscopy measurements are coupled in our 3-tier strategy.

Material and method

A-tier; The 3D cell microenvironment is a dynamic ecosystem that exhibits profound cellular heterogeneity as well as complex interactions. Soft agar, spheroids, and hanging drops were used. Cell phenotypes have been characterized and next, their spatial organization and inter-cellular cross-talk have been reconstructed.

B-tier; An *in silico* and *in vitro* platform has been set for medium-to-high throughput efficacy and ADMETox profiling addressing the pharmacokinetics, pharmacodynamics, and toxicokinetics (“hit-to lead” screening) via a proteogenomic perspective to account for the “actionable -ome”.

C-tier; For candidate companion biomarkers, we focus on the number, nature, and cargo of exosomes. We have established that cell phenotypes secrete “messages” mediating short- or long- range communication within the microenvironment and beyond. Exosomes also serve as a toolbox for drug resistance and drug-drug interactions.

Results and discussion

Our findings indicate that cellular heterogeneity and plasticity empowers drug repurposing as the 3D cell microenvironment is a key determinant of cell behaviour and function in (patio)physiology. Overall, exosomal profiles show distinct features.

Conclusion

Proteogenomic analysis empowers our pharmaco-centric strategy toward a better-informed druggable proteome with emphasis on proteoforms and/or their life-span for drug repurposing.

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Activation of Homologous Recombination and Non-Homologous End Joining after DNA damage in mouse oocytes

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Oocytes are amongst the most long-lived cells in the body. The oocytes formed in the ovaries during embryogenesis and remain arrested at Prophase I (GV oocytes) of meiosis. They can remain at this stage for a protracted period of time until they receive a stimulus to resume meiosis. This protracted state of arrest makes them extremely vulnerable in accumulating DNA damaging insults, which affect the genetic integrity of the female gametes and therefore the genetic integrity of the resulting embryo. The mechanisms that oocytes use in order to respond to DNA damaging insults is not yet fully understood. In our experiments, by using immunofluorescence and confocal microscopy, we try to determine the pathways that participate in Double Strand Break repair (DSBs), i.e. Homologous Recombination (HR) or Non-Homologous End Joining (NHEJ), and also the proteins and factors that may participate in these pathways. At the same time, we compare the repair capacity in oocytes between young (2 months old) and old (>6 months old) mice, underlying their differences. We observed that DNAPKcs, a key protein for NHEJ, is activated after potential damage in GV oocytes derived from both young and old mice, but the activation seems to be faster in the case of young mice. DNAPKcs may also has an important physiological role following entry into meiosis I, because it is detected in M-phase oocytes. Elevated levels of Rad51 in GV oocytes are also detected after damage, making HR a potential mechanism in their repair. Hence, it is essential that we understand the pathways that oocytes use for DNA repair. The use of an error prone mechanism, absent or inefficient repair could lead to infertility or chromosomal aberrations in the resulting embryo.

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A functional JNKs/ATM-p53 loop is necessary for the protection of dermal fibroblasts against UVB-induced apoptosis

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Although UVB radiation is mainly absorbed by the epidermis, approximately 5-10% of its photons reach and affect the upper part of the dermis. Physiologically relevant UVB doses, able to provoke erythema, induce apoptosis in human dermal fibroblasts in vitro, as well as in the dermis of SKH-1 mice. Given the sparse and even contradictory existing information on the effect of UVB radiation on dermal fibroblasts' viability, aim of this work was to unravel the crucial signaling pathways regulating the survival of UVB-treated human dermal fibroblasts. We found that UVB radiation immediately stimulates the phosphorylation of MAPK family members, as well as Akt, and is genotoxic leading to the delayed ATM-p53 axis activation. Akt phosphorylation after UVB radiation is EGFR-mediated and EGFR inhibition leads to a further decrease of viability, while the Akt activator SC79 rescues fibroblasts to an extent by a mechanism involving Nrf2 activation. The known Nrf2 activator sulforaphane also exerts a partial protective effect, although by acting in a distinct mechanism from SC79. On the other hand, inhibition of JNKs or of the ATM-p53 axis leads to a complete loss of viability after UVB irradiation. Interestingly, JNKs activation is necessary for p53 phosphorylation, while the ATM-p53 pathway is required for the long-term activation of JNKs and Akt, reassuring the protection from UVB. Although UVB radiation results in intense and prolonged increase of intracellular ROS levels, classical anti-oxidants, such as Trolox, are unable to affect Akt, JNKs or p53 phosphorylation and to revert the loss of fibroblasts' viability. Collectively, here we provide evidence that the main viability-regulating UVB-triggered biochemical pathways act synergistically towards the protection of human dermal fibroblasts, with EGFR/Akt and Nrf2 serving as auxiliary anti-apoptotic machineries, while JNKs/ATM-p53 activation and interplay being overriding and indispensable for the perpetuation of cellular defense and the maintenance of cell viability.

Reference

Mavrogonatou et al, *Cell Death Dis.* 2022 Jul 25;13(7):647. doi: 10.1038/s41419-022-05106-y.

P85

Nuclear translocation of SRPKs is associated with chemotherapeutic agents sensitivity in cancer cells

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Serine/arginine protein kinases (SRPKs) phosphorylate Arg/Ser dipeptide-containing proteins that play crucial roles in a broad spectrum of basic cellular processes. Here, we report that exposure of HeLa cells to DNA damage inducers triggers the nuclear translocation of SRPK1 and SRPK2. Furthermore, we show that nuclear SRPKs do not protect from, but on the contrary, mediate the cytotoxic effects of genotoxic agents, such as 5-fluorouracil (5-FU) and cisplatin. Confirming previous data showing that the kinase activity is essential for the entry of SRPKs into the nucleus, SRPIN340, a selective SRPK1/2 inhibitor, blocked the nuclear accumulation of the kinases, thus diminishing the cytotoxic effects of the drugs. ATM/ATR-dependent phosphorylation of specific residues within the spacer domain of SRPKs is essential for the redistribution of the kinases to the nucleus. Inhibition of ATR/ATM kinase activity by specific low-molecular weight inhibitors abolished nuclear localization of SRPKs and conferred tolerance to 5-FU and cisplatin treatment. These findings suggest that SRPKs may have a decisive role in coordinating cellular response to DNA damage.

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Pulmonary infection by SARS-CoV-2 induces senescence accompanied by an inflammatory phenotype in severe COVID-19: possible implications for viral mutagenesis

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SARS-CoV-2 infection of the respiratory system can progress to a multi-systemic disease with aberrant inflammatory response. Cellular senescence promotes chronic inflammation via senescence-associated secretory phenotype (SASP). We investigated whether COVID-19 disease is associated with cellular senescence and SASP. Autopsy lung tissue samples from 11 COVID-19 patients and 43 age-matched non-COVID controls with similar comorbidities were analysed by immunohistochemistry for SARS-CoV-2, markers of senescence and key SASP cytokines. Virally-induced senescence was functionally recapitulated *in vitro*, by infecting epithelial Vero-E6 cell line and a 3D alveosphere system of alveolar type 2 (AT2) cells with SARS-CoV-2 strains isolated from COVID-19 patients. SARS-CoV-2 was detected by immunocytochemistry and electron microscopy predominantly in AT2 cells. Infected AT2 cells expressed the angiotensin-converting-enzyme 2 (ACE2) and exhibited increased senescence (p¹⁶INK4A and GL13 positivity) and IL-1 β and IL-6 expression. *In vitro*, infection of Vero-E6 cells with SARS-CoV-2 induced senescence (GL13), DNA damage (γ -H2AX), increased cytokines (IL-1 β , IL-6, CXCL8) and



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Apolipoprotein B mRNA-editing (APOBEC) enzyme expression. Next-generation-sequencing analysis of progenies obtained from infected/senescent Vero-E6 cells demonstrated APOBEC-mediated SARS-CoV-2 mutations. Dissemination of the SARS-CoV-2-infection and senescence was observed in extra-pulmonary sites (kidney and liver) of a COVID-19 patient. We demonstrate that in severe COVID-19, AT2 cells infected by SARS-CoV-2 exhibit senescence and a proinflammatory phenotype. *In vitro*, SARS-CoV-2 infection induces senescence and inflammation. Importantly, infected senescent cells may act as a source of SARS-CoV-2 mutagenesis mediated by APOBEC enzymes. Therefore, SARS-CoV-2-induced senescence may be an important molecular mechanism of severe COVID-19, disease persistence and mutagenesis.

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Loss of the Fanconi anemia pathway is synthetic lethal with Geminin deficiency

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Accurate genome duplication is essential during somatic cell division to ensure the inheritance of a complete copy of parental DNA by the offspring. DNA licensing is a conserved mechanism ensuring that the genetic material is fully replicated whereby restricting single origin firing to once-per-cell-cycle. Licensing of already fired origins induces rereplication, a substrate of extensive replication stress and DNA damage. Interestingly, certain tumor types exhibit a hyperactive licensing machinery, associated to increased genomic instability and adverse prognosis in cancer patients. However, and despite abnormal licensing is a genomic trait of cancer, the downstream mechanisms signaling licensing defects and responsible for the resolution of rereplication-induced lesions are poorly characterized. To identify targetable pathways protecting cells with induced-rereplication, we performed an siRNA-high content screen in human TERT-immortalized retinal pigment epithelial (RPE1) cells transiently depleted of the licensing inhibitor Geminin. We identified a previously uncharacterized role of FANCD2 in signaling replication defects in cells undergoing origin relicensing, evidenced by FANCD2 recruitment on chromatin and foci formation early upon Geminin depletion. Loss of FANCD2 in Geminin-depleted cells increased fork velocity and single and double-strand breaks formation, with damaged cells arresting in G2 cell-cycle phase and ultimately facing cell death. Hypersensitivity of Geminin-depleted cells to FANCD2 loss was dependent on canonical Fanconi Anemia pathway activation. At the mechanistic level, our data suggest that FANCD2 prevents unrestrained fork progression in rereplicating cells. Specific compounds recently demonstrated in our group to impair licensing could provide a novel therapeutic strategy to target cancer cell survival in FANCD2-deficient cancers.

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Replication stress response during *in vitro* co-infection with *Helicobacter pylori* and *Escherichia coli*

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Persistent *Helicobacter pylori* (*Hp*) infection is as a major risk factor for gastric neoplasia. *Hp*-induced inflammation favors DNA damage and mutation through ROS production, dependent on the modulation of DNA damage repair (DDR) systems. Clinical outcome of gastritis involves interplay between host genetic predisposition, bacterial virulence components and environmental factors. Gastric lesions in *Hp*-positive patients have been related to an altered composition of gut microbiota compared to healthy subjects. The enteropathogen *Escherichia coli* (*Ec*), associated to colorectal cancer, is a prevalent co-inhabitant of *Hp* in the same host.

Having already investigated the putative effects on genomic instability induced by *Hp* infection on gastric epithelial cells (Kontizas et al. 2020), we extended our studies to an *in vitro* *Hp* and *Ec* co-infection model. To this end, human gastric epithelial GES-1 cells were sequentially co-infected with *Hp* and *Ec* strains (7.13 and UT189, respectively). RNA-Seq on poly(A)-enriched transcripts and Differential Expression Analysis (DEA) suggested a significant deregulation of 74 genes during co-infection, 125 genes during *Hp* infection, and 50 genes during *Ec* infection alone. Significantly deregulated genes were subjected to pathway over-representation analysis, indicating a putative deregulation of a number of biological processes associated with carcinogenesis, including NF-κB and IL-17 signaling. Phosphorylated histone H2AX (γH2AX), a characteristic marker of double strand break formation, was shown to be upregulated in response to *Hp* infection. With regards to DDR mechanisms, pathway-level differential abundance analysis (Fry) showed a significant upregulation of Nucleotide Excision Repair during infection with *Hp* or *Ec* alone. Key components of interest involved in DDR were further examined on a protein level, under the same co-infection protocol. FEN1 and RAD51 effector proteins of Base Excision Repair and Homologous Recombination respectively, were found downregulated during *Hp* and *Ec* co-infection or *Hp* infection alone, suggesting involvement of bacterial co-infection in gastric carcinogenesis.

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SR Protein Kinase 1 Inhibition by TAF15

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Although SRPKs were discovered nearly 30 years ago, our understanding of their mode of regulation is still limited. Regarded as constitutively active enzymes, known to participate in diverse biological processes, their prominent mode of regulation seems to mainly depend on their intracellular localization. Molecular chaperones associate with a large internal spacer sequence that separates the bipartite kinase catalytic core and modulate the partitioning of the kinases between the cytoplasm and nucleus. Besides molecular chaperones that function as anchoring proteins, few other proteins were shown to interact directly with SRPK1, the most-studied member of SRPKs, and alter its activity. In this study we identified TAF15 that has been involved in transcription initiation, splicing, DNA repair and RNA maturation, as a novel SRPK1-interacting protein. The C-terminal RGG domain of TAF15 was able to associate with SRPK1 and downregulate its activity. Furthermore, overexpression of this domain partially relocalized SRPK1 to the nucleus and resulted in hypophosphorylation of SR proteins, inhibition of splicing of a reporter minigene and inhibition of Lamin B Receptor phosphorylation. We further demonstrated that peptides comprising the RGG repeats of nucleolin, HNRPU and HNRNPA2B1 were also able to inhibit SRPK1 activity, suggesting that negative regulation of SRPK1 activity might be a key biochemical property of RGG motif-containing proteins.

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Inhibition of hyaluronan dephosphorylates ribosomal protein S6 in triple-negative breast cancer cells

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Hyaluronan is a predominant component of the extracellular matrix. It is synthesized on the plasma membrane by the hyaluronan synthases (HAS 1, 2 and 3). Hyaluronan exerts size-specific actions and influences various cellular functions, including cell proliferation, differentiation, migration and invasion, through its interactions with surface receptors such as CD44. Ribosomal protein S6 (rpS6) is a central constituent of the higher eukaryotic 40S ribosomal subunit which is activated upon phosphorylation at five serine residues in response to a variety of mitogens. Phosphorylation of rpS6 is associated with cell cycle progression, while it promotes the expression of additional ribosomal proteins and elongation factors that are necessary for protein translation. Studies in metastatic triple negative breast cancer (TNBC) cells have revealed HAS2 as the main enzyme responsible for hyaluronan biosynthesis in these cells. In order to investigate the possible role of hyaluronan in the phosphorylation and activation of rpS6, we treated MDA-MB-231 and Hs578T TNBC cells with 4-methylumbelliferone (4-MU), the only established inhibitor of hyaluronan biosynthesis. The results showed that the 4-MU reduced the phosphorylation status of rpS6 followed by the substantial suppression of breast cancer cell proliferation and growth. Treatment with 4-MU induced cell cycle arrest, as evidenced by FACS analysis, and reduced protein levels of cyclin D1, which has central roles in the regulation of cell cycle progression. Exogenous hyaluronan did not restore the phosphorylation of rpS6 indicating that the observed inhibitory effect of 4-MU was due to endogenously produced hyaluronan. Importantly, silencing of HAS2 in TNBC cells resulted in dephosphorylation of rpS6 in agreement with the hyaluronan biosynthesis inhibition results. These findings indicate a critical regulatory role for hyaluronan in metastatic breast cancer cell growth.

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Receptor Protein Tyrosine Phosphatase zeta 1 (RPTPZ1) regulates primary osteoblast proliferation and differentiation in vitro

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Receptor protein tyrosine phosphatase zeta 1 (RPTPZ1) is a member of the type V subfamily of receptor type protein tyrosine phosphatases (RPTPs). It is expressed mainly in the brain but also in endothelial cells, cancer cells and fully differentiated osteoblasts. DNA microarray analysis of primary osteoblasts showed that the gene encoding PTPRZ1 showed the highest level of induction during differentiation among 10,000 genes highlighting the receptor as a marker for terminally differentiated osteoblasts. In the same line, RPTPZ1 deficiency significantly enhanced osteosarcoma development in Trp53-heterozygous mice, suggesting that the receptor may act as an endogenous brake of osteoblast proliferation in vivo. In the present work, we used mice that are knockout for PTPRZ1 (Ptpz1^{-/-}) and their corresponding wild-type mice (Ptpz1^{+/+}) to isolate primary calvaria osteoblasts and identify potential differences related to their proliferation rate and differentiation. Ptpz1^{-/-} osteoblasts have an enhanced rate of proliferation compared to Ptpz1^{+/+} osteoblasts and elongated mitochondria. Under osteogenic differentiation conditions, Ptpz1^{-/-} osteoblasts have a stronger differentiation capacity into mature osteoblasts and an increased mineralization potential compared to Ptpz1^{+/+} osteoblasts, as estimated by alkaline phosphatase expression and calcium salts deposition. Similarly, a selective PTPRZ1 tyrosine phosphatase inhibitor enhanced osteoblasts differentiation. The expression of osteoblast specific marker genes, such as collagen type I and Runx2, and of members of the RANKL/OPG pathway will be discussed, also in relation to the rate of maturation of the bones and vertebrae in Ptpz1^{-/-} compared to Ptpz1^{+/+} mice in vivo. Collectively, our data suggest that PTPRZ1 significantly regulates osteoblasts functions through mechanisms that are being investigated to identify potential means of therapeutic interventions.

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The role of the membrane androgen receptor OXER1 in androgen induced calcium changes

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In prostate cancer, calcium homeostasis plays a significant role in the disease's development and progression. Intracellular calcium changes are an important secondary signal, triggered by a variety of extracellular stimuli, that controls many cellular functions. One of the main events affecting calcium is androgen signaling. Androgens can induce rapid calcium increases, mainly independently of the classical androgen receptor. Several studies have reported an effect mediated via G protein-coupled membrane receptors. In the present work, we have explored the role of OXER1 (a receptor of 5-oxo-EETE-arachidonic acid metabolite and a membrane androgen receptor, as we have previously reported in intracellular, androgen-induced, calcium increases in prostate cancer cells. Moreover, we report the specific signaling cascade(s) involved. Calcium was assayed using Fura 2-AM and/or Fluo-4-AM. Specific siRNAs and OXER1 agonists (5-oxo-EETE) and antagonists (GUE-1654) for OXER1 involvement. Downstream signaling was identified using specific kinase inhibitors and siRNAs. OXER1 expression was assayed by qPCR. Treatment of DU-145 cells with testosterone-BSA (a membrane impermeable analog) rapidly increased intracellular calcium, mainly from intracellular stores (as shown by nifedipine and U73122 -an inhibitor of L-type Ca²⁺ channel and phospholipase C, respectively). This effect was mediated by a GPCR (pertussis toxin-inhibited). The involvement of OXER1 was verified by OXER1 silencing and GUE-1654 inhibition. Surprisingly 5-oxo-EETE also specifically and dose dependently reverted the effect of testosterone-BSA. Additionally, it was found that both G_{ai} (without cAMP signaling) and G_{βγ} signaling via PI3K/Akt, FAK, c-Src and RACK-1 have a critical role in testosterone effect. Our findings clearly indicate OXER1 as the GPCR receptor involved in testosterone-induced calcium changes by activating specific G_α/G_{βγ} signaling cascade(s), and illustrate, once again, an important interaction between androgens and lipid metabolites for tumor cell fate regulation.

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Evaluating the action of sulfated hyaluronan in conventional 2D cultures and 3D spheroids of breast cancer cells

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Breast cancer constitutes one of the most common malignancies, with the expression patterns of estrogen receptors (ERs) being crucial during disease development. Hyaluronan (HA) is an extracellular matrix glycosaminoglycan (GAG) that plays a central role in a variety of biological processes. While HA is the only GAG not normally substituted with sulfate groups, sulfated hyaluronan (sHA) has previously been used in scientific studies showing promising anticancer action. The aim of the present study was to evaluate the effects sHA fragments have on breast cancer cells with different ER status in 2D and 3D cultures. To this end, ER α + MCF-7 cells, ER α -/ER β + MDA-MB-231 cells, and their ER β knockdown counterpart (clone shER β MDA-MB-231 cells) were treated with both non-sHA fragments and sHA fragments of 50 kDa. Proliferation, wound healing, adhesion and invasion assays were performed in order to determine the effects these HA fragments have on the cells' functional properties. The expression of matrix effectors was analyzed at a gene level using real-time PCR, and at the protein level using immunofluorescence and western blot analysis. Additionally, cell morphology and spheroid formation potential were examined. According to the results, sHA attenuates breast cancer cell proliferation, migration and invasion, while increasing their adhesion on collagen type I. Notably, the sHA fragments seem to exhibit a stronger effect on these properties compared to the one mediated by the non-sHA of the same molecular size, with the effect also dependent on the ER status. Moreover, the functional properties observed are corroborated with and explained by differences in mRNA and protein levels for matrix effectors and EMT markers. Consequently, a deeper understanding of the mechanism by which the sHA fragments orchestrate these processes could contribute to the development of therapeutic strategies.

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Human L-Dopa decarboxylase (DDC) involvement in Apoptosis

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L-Dopa Decarboxylase (DDC) is a pyridoxal-requiring enzyme that catalyzes the decarboxylation of L-3,4-dihydroxyphenylalanine (L-Dopa) to Dopamine (DA). The biochemical functions of DDC in physiological and pathological pathways remain poorly understood. Additionally, the biological significance of the numerous human DDC isoforms is almost unknown. Our laboratory has demonstrated that Annexin V, an apoptosis marker, inhibits L-Dopa decarboxylase activity in humans. Work from our research team has also shown that DDC, as well as the truncated DDC isoform, Alt-DDC, interact with Annexin V in human cells and tissues. In the present study, the possible role of DDC in apoptosis was further explored. Using shRNA-mediated RNAi, DDC expression was knocked down in human cancer cells. Apoptosis was detected and measured using the diphenylamine method. Protein expression was analyzed by western blotting. Our results indicated that DDC knockdown resulted in a threefold increase in apoptotic cell death. Additionally, DDC knockdown cells showed increased expression of important apoptotic markers, namely, Bak, Caspase-3, BCL-2, and Annexin V. The findings of this study extend our previous work and further support DDC's involvement in the biochemical pathways leading to apoptotic cell death. Our data underline the emerging importance of the DA-synthesizing enzyme in key biological processes.

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An alternative spliced transcript of human ribonuclease κ gene is involved in cell fate under oxidative stress

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Oxidative stress leads to cell damage because of the imbalance between production and accumulation of oxygen reactive species and cell ability to detoxify. The cellular stress response involves a decision between survival or cell death. Ribonucleases (RNases) are enzymes involved in many biological processes. Although most of them play key roles in the maturation, quality control and turnover of cellular RNAs, recent studies have shown that some RNases are activated in response to stress [1]. Human RNase κ belongs to a highly conserved family in all metazoans, which is expressed in almost all tissues [2,3]. These observations in combination with the identification of a variety of alternative spliced transcripts in a wide panel of human cell lines [4], suggest its implication in different biological functions that are currently under investigation. Among them, RNase κ is required for the internalization of diverse acid-dependent viruses [5], is engaged in piwi RNA production [6], enhances interferon secretion and promotes apoptosis [7].

The aim of this study was to investigate the involvement of RNase κ to the oxidative stress cell response. For this purpose, HEK293T cells were exposed to different concentrations of H_2O_2 for several incubation periods. After confirmation of induction of cellular and apoptotic death, the expression levels of RNase κ basic transcript (01) and its alternative transcripts 7, 9, 14 and 20 were determined by RT qPCR. Our data clearly demonstrated that although H_2O_2 induced oxidative stress does not affect the RNase κ expression levels of transcripts 01, 9, 7 and 20, it causes a significant increase (up to 10-fold) of RNase κ transcript 14, in a wide range of H_2O_2 concentrations. Additionally, the finding that the expression levels of transcript 14 were also increased in the presence of the proteasome inhibitor Carfilzomib, reveals its significant role in cellular and apoptotic death.

- ¹ Protective Effects of Recombinant Human Angiogenin in Keratinocytes: New Insights on Oxidative Stress Response Mediated by RNases. (2022) Culurciello R. et al. *Int. J. Mol. Sci.*, 23, 8781.
- ² Molecular cloning and characterization of the human RNase κ , an ortholog of Cc RNase. (2007) Economopoulou M. et al. *Nucleic acids research* 35 (19), 6389-6398.
- ³ Genomic structure and expression analysis of the RNase κ family ortholog gene in the insect *Ceratitis capitata* (2008) Rampias T.N. et al. *The FEBS journal* 275 (24), 6217-6227.
- ⁴ Identification of novel alternative transcripts of the human Ribonuclease κ (RNASEK) gene using 3' RACE and high-throughput sequencing approaches. (2020) Adamopoulos P.G. et al. *Genomics* 112(1), 943-951.
- ⁵ RNASEK is required for internalization of diverse acid-dependent viruses. (2015) Hackett B.A. et al. *Proc Natl Acad Sci U S A*. 112(25):7797-802.
- ⁶ RNase κ promotes robust piRNA production by generating 2', 3'-cyclic phosphate-containing precursors. (2021) Shigematsu M. et al. *Nat Commun.*, 12: 4498.
- ⁷ Fish Paralog Proteins RNASEK-a and-b Enhance Type I Interferon Secretion and Promote Apoptosis. (2021) Sun Z-C et al. *Front. Immunol.* 12:762162.

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A novel function of LONP-1 protease in mitochondrial retrograde response

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Mitochondrial damage leads to accumulation of misfolded/unfolded proteins and activation of stress responsive pathways to cope with proteotoxic stress. The highly conserved ATP-dependent mitochondrial LONP1 protease is an important regulator of mitochondrial protein homeostasis, acting both as a chaperone and a protease. We employed here a null mutant *Caenorhabditis elegans* to study how deficiency of the lonp-1 gene affects the organismal responses in the nematode. We have shown that LONP-1 deficiency results in short-lived animals with disturbed mitochondrial network and excessive ROS production. These defects initiate an adaptive cellular signaling and induce the Antioxidant Stress Response, the Heat Shock Response and the mitochondrial Unfolded Protein Response (UPR^{mt})¹. Under conditions of mitochondrial stress, UPR^{mt} activity normally restricts the activation of a p38/MAPK (MAPK^{mt}) mitochondrial surveillance pathway, consisting of the DLK-1/SEK-3/PMK-3 signaling cascade^{2,3}. Induction of MAPK^{mt} under mitochondrial respiratory dysfunction is triggered downstream of the Mitochondrial Associated Degradation (MAD) pathway³. The MAD pathway mediates export of ubiquitinated proteins, through the CDC-48/UFD-1/NPL-4 protein complex of the Mitochondrial Outer Membrane (MOM), and transfers them to the proteasome for degradation⁴. These molecular interactions indicate the presence of extensive crosstalk between cellular surveillance mechanisms following mitochondrial stress. To gain insight into these quality control mechanisms, we investigated the impact of LONP-1 deficiency on the activation of and interplay between the associated pathways. In this study, we have demonstrated that the MAPK^{mt} pathway is induced in lonp-1(ko) mutants, even though they exhibit increased activity of ATFS-1, a major transcription factor of UPR^{mt}. However, knock down of atfs-1, further upregulates MAPK^{mt}, supporting the restrictive role of UPR^{mt} on MAPK^{mt} induction. Furthermore, we currently focus on the interaction of MAD and MAPK^{mt} pathways in lonp-1(ko) mutants, after silencing key components of the MAD pathway in transgenic animals expressing an established marker of MAPK^{mt} cascade.

References

- 1 Taouktsi, E.; Kyriakou, E.; Smyrniotis, S.; Borbolis, F.; Bondi, L.; Avgeris, S.; Trigazis, E.; Rigas, S.; Voutsinas, G. E.; Syntichaki, P, Organismal and Cellular Stress Responses upon Disruption of Mitochondrial Lonp1 Protease. *Cells* 2022, 11, (8).
- 2 Tjahjono, E.; McAnena, A. P.; Kiriienko, N. V., The evolutionarily conserved ESRE stress response network is activated by ROS and mitochondrial damage. *BMC Biol* 2020, 18, (1), 74.
- 3 Munkacsy, E.; Khan, M. H.; Lane, R. K.; Borror, M. B.; Park, J. H.; Bokov, A. F.; Fisher, A. L.; Link, C. D.; Rea, S. L., DLK-1, SEK-3 and PMK-3 Are Required for the Life Extension Induced by Mitochondrial Bioenergetic Disruption in *C. elegans*. *PLoS Genet* 2016, 12, (7), e1006133.
- 4 Liao, P. C.; Wolken, D. M. A.; Serrano, E.; Srivastava, P.; Pon, L. A., Mitochondria-Associated Degradation Pathway (MAD) Function beyond the Outer Membrane. *Cell Rep* 2020, 32, (2), 107902.

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A promising circulating tumour cell model for the evaluation of the anti-tumour effects of artesunate

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Artesunate (AS), a known antimalarial agent, has been lately appreciated for its anti-tumour activity in a plethora of cancer types. Circulating tumour cells (CTCs) are shed by the tumor into the bloodstream, overcome numerous difficulties to survive, exhibit resistance to certain drugs and eventually give rise to metastases in distant organs. Recently, AS has been shown to decrease JunB expression, which is also involved in the metastatic cascade. The aim of the study is to examine the effect of AS on non-adherent cell culture models, which resemble CTCs.

Breast (MDA-MB-231 and MDA-MB-436), lung (A549, H1299 and H1650), and colon (HT-29 and SW-620) cancer cell lines were evaluated. Establishment of the CTC model was achieved by poly(2-hydroxyethyl methacrylate). The equivalent adherent cell cultures and a patient-derived colon CTC-MCC-41 cell line was used for side-by-side comparisons. Cell viability was examined following treatment with different concentrations of AS. Migration was examined by the wound healing assay and JunB expression was assessed by western blot.

AS decreased viability of all adherent cell lines in a concentration-dependent manner; IC_{50} was shifted to the right in the non-adherent cells. AS (10 μ M, 48 h) inhibited viability of all adherent cell lines; the effect was less prominent in the non-adherent model. AS effects were more pronounced on lung and colon, compared to breast cancer cells. Interestingly, the effect on CTC-MCC-41 was a decrease of the viability in a very promising level ($46\% \pm 6$), implying that AS could be effective on disseminating cancer cells. Moreover, AS decreased cell migration and JunB expression in adherent cells.

Antitumor agents might exert different effects on tumor cells depending on their adherent status and tissue origin. Evidently, the present model, whereby the free-floating cells mimicking CTCs, is promising in identifying differences in response to AS compared to the attached counterparts.

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P98

Pharmacological characterization of first-generation catalytic PTEN inhibitors *in vitro*, *in cellulo* and *in vivo*

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Protein/lipid phosphatases have been largely unexplored towards pharmacological targeting, compared to kinases. PTEN, a prominent lipid phosphatase that regulates the PI3K/Akt/mTOR signaling pathway is implicated in several human diseases and pathologies. However, there is limited information on PTEN modulators, while targeting PTEN catalytic activity or interaction with membranes with small molecules has been proven difficult. In the present study, we have revisited and comprehensively characterized currently used 1st generation PTEN inhibitors, bisperoxo-vanadium (V) complex (bpVs) compounds, *in vitro* and *in vivo*.

BpVs exhibit variable inhibition (μM levels) of PTEN activity assayed with water-soluble PI(3,4,4)P3 *in vitro*, that depends on the reducing conditions. In cells, PTEN inhibition by the bpV compounds results in downstream activation of Akt and mTORC1 in a PTEN-dependent manner. However, bpV compounds increase also Erk1/2 phosphorylation in a PTEN-independent manner. Our results do not confirm the oxidative inhibition of PTEN in cells. *In vivo*, subchronic administration of bpVs in Wistar rats activates mTOR pathway in peripheral tissues, most notably kidney and also suppresses free motility and exploratory behavior of animals in the open field test.

The use of 1st generation PTEN inhibitors, especially bpVs, is widespread, but currently there is no consensus regarding their mechanism of action or safety profile. In our ongoing studies, we are using *in vivo/cellular* assays to understand the mechanism of action of the already existing PTEN modulators, while our long-term goal is to clarify the importance of pharmacological targeting of PTEN and develop a pattern for the design of new potential drugs.

P99

Adrenergic receptors and pleiotrophin in endothelial cells function *in vitro*

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Adrenergic receptors (ARs) have a wide distribution and affect numerous functions, including angiogenesis. Catecholamines, norepinephrine and epinephrine, are the endogenous ligands of ARs and induce proliferation and migration of endothelial cells, thus promoting angiogenesis. Pleiotrophin (PTN) is a critical growth factor that regulates diverse physiological functions, among which angiogenesis. PTN has been shown to act by interacting with numerous cell surface receptors, such as PTPRZ1, $\alpha\text{v}\beta\text{3}$ integrin, nucleolin and VEGFR2 and to increase phosphorylation of downstream effectors, thereby activating the signal transduction related to cell growth, migration, and tube formation of endothelial cells. PTN has been also shown to mediate actions of amphetamines in the nervous system, and this indicates its possible interplay with the adrenergic system. In this study, we investigate the impact of adrenergic receptors in endothelial cells and the involvement of PTN in this pathway, using endothelial cells isolated from human umbilical vein (HUVEC) or lungs (LMVEC) of Ptn^{wt}/Ptn^{ko} and PTPRZ1^{wt}/PTPRZ1^{ko} mice. The cells are cultured in the presence or absence of β adrenergic agonists and/or antagonists and we examine cell functions, such as proliferation, migration, and activation of downstream signaling pathways. β ARs agonists induce proliferation and migration of endothelial cells but their effect is significantly smaller in Ptn^{ko} compared to Ptn^{wt} endothelial cells. Similarly, the effect of β adrenergic antagonists is smaller in Ptn^{ko} compared to Ptn^{wt} endothelial cells. The phosphorylation of ERK1/2 is also enhanced by the agonists and inhibited by the antagonists of the β ARs. Interestingly, the antagonists inhibit some but not all observed effects of the agonists, suggesting that besides β ARs, other mechanisms may be involved. In conclusion, our data suggest that ARs regulate endothelial cells functions and PTN may be implicated in their signaling.

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P100

WISP-1 regulates collagen turnover by airway smooth muscle cells differentially in asthma and COPD

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Asthma and COPD are chronic inflammatory diseases characterized by airway remodeling. WISP-1 is a target gene of WNT/b-catenin signaling that has different roles in asthma and COPD. WNT/b-catenin signaling promotes asthma pathogenesis, while it may have a protective and regenerative role in COPD. The aim of this study was to explore the effect of WISP-1 on collagen synthesis and deposition in the airways of patients with asthma and COPD.

Protein and mRNA expression of WISP-1 was assessed in endobronchial tissue biopsies from patients with asthma and COPD who underwent bronchoscopy. Primary cultures of airway smooth muscle cells (ASMC) were established from endobronchial tissue biopsies obtained from patients with asthma and COPD and stimulated with rhWISP-1. Gene and protein expression of matrix metalloproteinase (MMP)-1, tissue inhibitor of MMP (TIMP)-1 and collagenous proteins was assessed by qPCR, ELISA and immunofluorescence. Collagenous proteins were also measured by the sircol assay.

Gene and protein expression of WISP-1 was significantly higher in endobronchial tissues from patients with asthma as compared with COPD. WISP-1 stimulated gene and protein expression of MMP-1 in ASMC and this effect was more prominent in asthma than in COPD. WISP-1 had no effect on gene or protein expression of TIMP-1 neither in asthma nor in COPD. In ASMC from patients with asthma WISP-1 significantly stimulated gene and protein expression of COLA1, whereas in ASMC from patients with COPD WISP-1 decreased COLA1 expression.

These results indicate that there is a differential effect of WISP-1 on the turnover of collagenous proteins by ASMC in asthma and COPD and provide new insights in the understanding of the role of WNT signaling pathway in airway remodeling in these diseases.

P101

Moesin negatively regulates endothelial cell migration through interaction with $\alpha_v\beta_3$ integrin and inhibition of c-Met

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Moesin is a member of the ERM (Ezrin, Radixin, and Moesin) protein family, acts as a cross-linker between cell membrane-anchoring proteins and actin-based cytoskeleton and is implicated in cell-cell recognition, epithelial morphogenesis, cell adhesion, and migration. MALDI-TOF and Western blot analyses, as well as immunofluorescence and proximity ligation assays identified moesin as an $\alpha_v\beta_3$ -interacting molecule in human endothelial cells. Down-regulation of moesin expression or decreased moesin- $\alpha_v\beta_3$ interaction because of vascular endothelial growth factor A or pleiotrophin stimulation, resulted in significant enhancement of cell migration, which was abolished by pharmacological inhibitors of c-Met, ERK1/2 kinases, and cell surface nucleolin, but not upon specific inhibition of VEGF receptor 2. In agreement, overexpression of moesin in endothelial cells resulted in inhibition of cell migration, verifying its negative regulatory effect. We have recently shown that in endothelial cells that are knockout for PTPRZ1, β_3 integrin expression is decreased and cell migration is significantly enhanced due to c-Met activation. In these cells, downregulation of moesin by siRNA did not further stimulate cell migration, suggesting that downregulation of PTPRZ1 or moesin result in activation of endothelial cell migration through the same c-Met-dependent pathway. Altogether, these data uncover a novel partner of $\alpha_v\beta_3$ that regulates endothelial cell migration, help explain the multifaceted role of $\alpha_v\beta_3$ on angiogenesis and identify potential new targets for the development of novel anti-angiogenic therapeutic approaches.

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P102

Development of a novel in vitro assay for the identification of chemical inhibitors of the MYC/MAX protein complex

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Development of new anti-cancer drugs for targeted therapy has been the subject of intense study during the last decades. In this regard, a promising target for anticancer treatment is the MYC oncoprotein, which is encoded by the proto-oncogene MYC. The MYC protein initiates transcription of target genes, after forming a complex with MAX¹. Binding of MYC to MAX occurs through the basic helix-loop-helix-leucine zipper (bHLHLZ) domain, thereby inducing structural changes that are necessary for binding to DNA and initiation of transcription². Thus, small molecules acting specifically on the bHLHLZ domain of MYC, aiming at inhibiting the formation of the complex between MYC and MAX, are very promising as anticancer drugs.

We report the development of a novel in vitro, cell-free assay, which assesses quantitatively the amount of complex between MYC and MAX and can be used for screening of new inhibitors against MYC. The assay is based on the pull-down principle, using reagents and infrastructure that are common or easily accessible. To establish the assay, we tested different tags (GST or 6His) for the expression, purification and complex formation between MYC and MAX, as well as different conditions and relative amounts of the two proteins. From these tests we found that the optimal conditions for complex formation require pull-down of 6His-MAX with GST-MYC that was preloaded on beads. Interestingly, using ImageJ, we show that the assay determines quantitatively the amount of formed complex, based on the assessment of levels of His-MAX bound to GST-MYC-beads. Additionally, by testing known inhibitors of MYC, such as 10058-F4 and Mycro3, we provide proof-of-principle that the assay can be used for screening newly designed and synthesized compounds. Our data demonstrate the significance of this assay in providing necessary mechanistic evidence that a new drug, under development, indeed acts by disruption of the MYC/MAX transcriptional complex.

References

- ¹ Dang C. V. (2012). MYC on the path to cancer. *Cell*, 149(1), 22–35.
- ² Beaulieu, M. E., Castillo, F., & Soucek, L. (2020). Structural and Biophysical Insights into the Function of the Intrinsically Disordered Myc Oncoprotein. *Cells*, 9(4), 1038.

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P103

Blocking Nanobodies as potential “safety switches” in T-lymphocytes

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The selective and controllable inhibition of T-cell function is crucial not only for the design of personalized treatments against T-cell-dependent pathologies, but it can also comprise an invaluable tool in the fields of organ transplantation and T-cell immunotherapies. Hence intense efforts are focused on identifying compounds capable of manipulating T-cell activation. Lck, a member of the Src family of protein tyrosine kinases (SFKs), is mandatory for T-cell signaling initiation and subsequently T-cell activation and has become an attractive target for the production of small molecule inhibitors. However, the development of highly selective and potent Lck inhibitors has not been met with success, due to an astonishing structural homology shared by SFK members within their catalytic centers. The current project aims to achieve selective downregulation of T-cell signaling and T-cell function via specific inhibition of Lck, using intracellularly expressed nanobodies (Nbs).

These Nbs are designed to recognize a poorly conserved regulatory region of Lck and will act in endosomal compartments in order to prevent the translocation of Lck into the plasma membrane and immunological synapse, the natural locations of Lck activity. This alteration in Lck traffic should block the initiation of T-cell signaling and downregulate T-cell function.

A preliminary screening of different Nbs was accomplished by transfecting HEK293T cells with Lck in the presence or absence of Nbs. The Nbs that were capable to bind Lck were further transduced in T-cell lines and it was tested the ability of Nbs to create a binding complex with the endogenous Lck, using Co-Immunoprecipitation experiments and confocal microscopy.

Obtained data revealed at least one Nb capable of binding Lck intracellularly. Our next goals include the evaluation of Nbs' ability to attenuate T-cell signaling as well as the specificity of Nb by testing its activity in different cell lines which express other SFK members.

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Με τη συγχρηματοδότηση της Ελλάδας και της Ευρωπαϊκής Ένωσης

P104

eIF6-linked regulation of translation and signaling in vemurafenib resistant melanoma cell lines

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Melanoma is the most aggressive type of skin cancer, characterized by high metastatic potential and its development is affected by the presence of both hereditary and environmental factors. The most common mutation in melanoma is BRAF^{V600E}, which leads to excessive downstream signaling and translational deregulation. Targeting of BRAF^{V600E} using specific small molecule inhibitors, such as vemurafenib, is widely used for treatment of metastatic melanoma. However, malignant cells rapidly develop resistance to targeted therapy. eIF6 was recently reported as a promising diagnostic and prognostic biomarker for poorer survival of melanoma. Possessing both ribosomal anti-association and 60S-biogenesis roles, eIF6 is overexpressed in many cancer types. Participation of eIF6 in the development and acquired resistance of melanoma cells remains to be elucidated. In the present study, we developed BRAF^{V600E}-mutated vemurafenib resistant (VR) A375 and SK-MEL5 cell lines. eIF6 was differentially expressed in the VR cells and its nucleus/cytoplasm localization ratio was altered. The 60S:40S, though, was not affected, as indicated by ribosome profiling using sucrose density gradients. To further investigate its role in translation regulation and signaling in melanoma, we constructed eIF6-stably expressing A375 and SK-MEL5 cell lines. eIF6-overexpressing A375 cells, mainly showed a slight decrease in the phosphorylation of S6 kinase, while metastatic SK-MEL5 cells showed a significant increase in the phosphorylated forms of AKT, S6K and ERK kinases and a decrease of the phospho-mTOR (S2448) kinase. Both cell lines showed higher expression of total and phospho-eIF4E, indicating enhanced cap-dependent translation. Puromycin staining revealed increased global translation rates only in eIF6-overexpressing A375 cells. Basal levels of eIF6 were also checked in four genetically engineered mouse melanoma cells lines, showing increased expression of eIF6 in two of them. Collectively, our results suggest a significant role of eIF6 in both vemurafenib resistant melanoma cell lines by affecting major signaling events and cap-dependent translation regulation.

P105

In vitro evaluation of Mavrodaphne grape stem extract as potential anti-inflammatory agent in endothelial cells

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Background: Grape stems constitute an abundant byproduct of the winemaking process with serious polluting properties. It has been reported that grape stems are a rich source of bioactive phytochemical compounds, namely polyphenols including resveratrol, catechin, procyanidin B3 and gallic acid, which are detected in higher concentrations in stems compared to grapes or wine.

Methodology: Grape stem extract was prepared from the native Greek vine variety Mavrodaphne, as described elsewhere. The total polyphenolic and total flavonoid contents had been previously quantified using HPLC analysis and UPLC-MS/MS analysis¹. The main objective of the current study was to investigate the potential anti-inflammatory effect of the polyphenol-rich extract derived from grape stems of Mavrodaphne variety. Initially, human umbilical vein endothelial cells (HUVEC) were treated with increasing concentrations (0.1 – 30 ug/mL) of extract to assess the effect on cell viability after 24 h and 48 h of treatment, using MTT-based chromogenic assay. Interleukin-6 (IL-6) levels were determined in the supernatant of HUVECs pre-treated with extract and stimulated with TNF α using ELISA. Gene expression analysis of adhesion molecules VCAM-1 and ICAM-1 was performed by quantitative RT-PCR, while the activation of p38 and NF- κ B signaling cascades through phosphorylation of p38 and Inhibitory kappa Ba (I κ Ba) kinases, respectively, was evaluated by Western blot analysis.

Results: Our results showed that administration of grape stem extract did not impair cell viability in the given experimental conditions. Furthermore, treatment with 5 or 10 ug/mL of grape stem extract alone did not induce any of the abovementioned pro-inflammatory mechanisms examined in this study, whereas pre-treatment with 10 ug/mL of extract significantly down-regulated the secretion of IL-6 by HUVECs in the presence of TNF α stimulus (p<0.05). Mavrodaphne grape stem extract effect on VCAM-1 and ICAM-1 expression, as well as on p38 and I κ Ba phosphorylation, in TNF α -stimulated HUVECs is still in progress.

References

- ¹ Veskoukis, A. S. et al. Grape stem extracts from three native Greek vine varieties exhibit strong antioxidant and antimutagenic properties. *Anticancer Res* 40, 2025–2032 (2020).

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P106**The importance of GemC1 during murine hippocampal development**

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The murine hippocampus is an intricate and continuous brain structure, located in the inner region of the mammalian temporal lobe. It is composed of two interlocking parts: the Cornu Ammonis (CA) and the Dentate gyrus (DG). In the latter, resides the subgranular zone (SGZ), a narrow layer of cells that acts as one of the two major sites of adult neurogenesis in the brain. Hippocampal abnormalities, whose molecular mechanisms have yet to be fully elucidated, have been associated with several neurological diseases and disorders, thus highlighting the hippocampus's major role in memory and learning.

Our research focuses on investigating whether the absence of GemC1, a member of the Geminin superfamily, affects the development of the murine hippocampus. To achieve this goal, we have generated GemC1 knockout mice. Thus far we have observed that GemC1 deficient mice show structural changes on the hippocampus both in embryonic and early postnatal mice. Concurrently, preliminary data show that the absence of GemC1 affects the expression of p73 in the developing hippocampus during embryogenesis. P73 has been shown to play a critical role in hippocampal development, since its replacement or depletion results in severe hippocampal dysgenesis, as shown in mouse models. Additionally, its transcriptional activation is regulated by GemC1, with the direct interactions between the two molecules affecting multiciliogenesis in mice.

Our analysis so far suggests that GemC1 portrays an important role in the formation of the murine hippocampus. We believe that these findings will help uncover the molecular mechanisms that govern hippocampal development and provide the necessary tools for the development of new and improved models for the study of hippocampus related diseases and disorders.

P107

Artificial bioscaffolds promote chondrogenesis in human Wharton's Jelly mesenchymal stem cells

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The hyaline cartilage is a connective tissue located on the articular surfaces of the bones providing resistance to compressive forces and low friction movement and consists mainly of abundant extracellular matrix (ECM) and chondrocytes. Due to the lack of blood vessels and nerves the ability of cartilage to regenerate and repair in cases of injury or disease as in osteoarthritis (OA), is limited. In recent years, tissue engineering has led to new techniques, with the manufacture of artificial biomimetic scaffolds using mesenchymal stem cell (MSCs) to repair and regenerate articular cartilage *in vitro*. Artificial scaffolds can support MSCs proliferation, ECM formation, and chondrogenesis. In the present study, we examined the differentiation of MSCs from Wharton's Jelly of human umbilical cord into chondrocytes in the presence of artificial scaffolds in the absence of chondrogenic differentiation medium. The scaffolds that were used are the type II collagen polypeptide and the synthesized elastin-mussel-silk-like polypeptide with a high number of lysines cross-linking between polypeptide chains, as well as their combination. The scaffolds evoked the differentiation of MSCs into chondrocytes as shown by the increased levels of the major matrix biomarkers of chondrogenesis, the morphological evaluation and Alcian Blue staining of the cultures. Moreover, the presence of scaffolds affected ERK1/2 and AKT signaling of MSCs. **Collectively, human Wharton's Jelly MSCs demonstrate the dynamics of chondrogenic differentiation, when cultured in the presence of crosslinked elastin-silk-mussel-like polypeptide scaffold, revealing its potential as a novel biomaterial in tissue engineering applications for the personalized treatment of OA.**

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Characterization of the Abracl expressing cell population in the developing mammalian forebrain

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Abracl (Abra C-terminal-like) is a non-typical winged-helix protein that shares similarity with the C-terminal Actin-binding domain 2 of the protein ABRA. The role of Abracl in the cell remains elusive so far, although it has been implicated in proliferation, migration and actin dynamics. In this study, we have analyzed the expression of Abracl protein in double immunofluorescence experiments with known markers; Ki67 for dividing cells, TUBB3 for post mitotic neurons, the subpallial-specific GABAergic-lineage-associated markers *Dlx2*, *Lhx6*, *Mash1* and *GAD65/67* and the cortical plate specific marker *Tbr1*. Our results show that during embryogenesis, Abracl is expressed in dividing cells of the subpallial subventricular zone as well as in subpallial-derived migrating interneurons and in post-mitotic neurons of the pallium. Notably, Abracl was also highly expressed in major fiber tracts of the forebrain, especially in the later stages of embryogenesis.

P109

Genetic interaction between histone acetylation writers and histone methylation readers in *Arabidopsis thaliana*

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Epigenetic mechanisms, such as histone post-translational modifications, play a critical role in regulating genomic function by fine-tuning gene expression patterns. GENERAL CONTROL NON DEREPRESSIBLE 5 (GCN5) is a histone acetyltransferase responsible for the acetylation of lysine residues on histone N-tails. GCN5 interacts with the transcriptional coactivator ADA2b, and mutations in GCN5 and ADA2b result in pleiotropic effects on every developmental aspect of the plant life cycle in *Arabidopsis thaliana*. Histone readers interpret histone tail modifications. EMSY-like 1 (EML1) and EMSY-like 2 (EML2) are members of a small family of transcriptional effectors in *Arabidopsis thaliana* that contain an Agetet/Tudor domain which has been characterized as a dual H3K4me3/H3K36me3 histone reader. EML1 and EML2 affect flowering time in *Arabidopsis thaliana*, while EML1 has also been associated with plant pathogen defence and normal seed development. To better understand the genetic interaction between histone acetylation writers and histone methylation readers, *eml1*, *eml2*, *eml1eml2* mutants were crossed with *gcn5-1* and *ada2b-1* mutants, and several aspects of the plant development were examined using morphological and physiological approaches. The double mutants *eml1ada2b-1* and *eml2ada2b-1* display novel phenotypes, including reduced hypocotyl length, abnormal cotyledon morphology, late flowering phenotype, increase in secondary rosette inflorescences and cauline leaves, and an extreme delayed and prolonged senescence. In contrast *eml1eml2ada2b-1* triple mutant plants exhibit a severe dwarf phenotype. The *eml1gcn5-1* and *eml2gcn5-1* mutants were phenotypically similar with the *gcn5-1* mutant, with a few differences, such as abnormal floral organ number and valve development, whereas these phenotypes were enhanced in the triple *eml1eml2gcn5-1* mutants. These results indicate that both EML1 and EML2 interact with GCN5 and ADA2b genes, and these genetic interactions and possible synergies are essential for normal plant development.

P110

Mcdas deficiency impairs ependymal cell differentiation in the mouse brain

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The subventricular zone constitutes one of the main neurogenic niches in the adult mouse brain. Ependymal cells surround the apical processes of neural stem cells that reside in subventricular zone, forming pinwheel structures. Most ependymal cells bear multiple motile cilia that beat coordinately to support cerebrospinal fluid flow in the ventricular system. A small subpopulation of ependymal cells bear two complex basal bodies that nucleate two cilia and are called bi-ciliated. However a lot of research is needed in order to unravel their developmental origin and the functional role they have.

Our lab research is focused on the molecular mechanism that coordinates the differentiation of embryonic neural stem cells towards the ependymal lineage. Previous findings from our research group have pointed out GemC1 and Mcdas, two members of Geminin superfamily, as master regulators of multiciliogenesis.

Aiming to unravel the role of Mcdas in ependymal cell differentiation, mice that constitutively lack Mcdas were generated. Mcdas-deficient mice are born in normal ratio but display growth retardation, develop hydrocephalus and die until the second week after their birth. In coronal brain sections we have seen that p73 and Foxj1, two transcriptional factors fundamental for multiciliogenesis, are expressed upon Mcdas deletion indicating that the progenitor cells are committed towards the ependymal cell fate. However, they do not manage to amplify their basal bodies and the differentiation of ependymal cells is disrupted. In whole mounts of the subventricular zone, only cells with one or two cilia have been detected.

In conclusion our data provide evidence that Mcdas is not implicated in the commitment of radial glial cells towards the ependymal lineage but has a fundamental role in later steps during the differentiation of ependymal cells.

P111

Developmental and cell-type specific expression of phospholipid phosphatase-related proteins in the CNS

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Phospholipid-phosphatase-related proteins (PLPPRs) are a family of neuron-enriched, developmentally-expressed membrane proteins that regulate glutamatergic synapses, filopodia, axonal branch formation, and growth cone navigation. PLPPRs are prone to heteromeric complex formation suggesting diversified effects on bioactive lipid and small GTPase signaling. Recent studies have focused on hippocampus and cortex early postnatal development, but PLPPRs expression in subcortical brain regions during development adulthood is far from known. Furthermore, there are no studies on co-expression of PLPPRs and their cell-type expression patterns. In the present study, our aim was to explore which brain regions and cell types express PLPPRs during development and adulthood.

For this purpose, we used quantitative PCR and Western blot for quantifying PLPPR mRNA and PLPPR3 protein expression in 5 tissues and 5 developmental stages. We also developed a custom computational screening tool to mine four publicly available mouse brain single-cell RNA-sequencing datasets (Allen Brain Atlas, mousebrain.org). Our qPCR analyses suggest ensuing expression of PLPPRs in subcortical brain areas, particularly in structures of the limbic system, which we have verified for PLPPR3 by western blotting. Single neuron expression analysis suggests high PLPPR co-expression in specific adult GABAergic interneuron as well as in cortical and hippocampal glutamatergic subtypes.

Our results indicate that PLPPRs are expressed at high levels in the adult limbic system, while GABAergic neurons show the highest degree of co-expression of PLPPRs. This points to a possible regulatory role of PLPPR heteromeric complexes in GABAergic neuron morphogenesis and function. Lastly, our computational screening approach for single cell sequencing datasets provides a tool to collect information about any gene and neuron type of interest.

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P112

Investigating the role of Abracl in a neuronal cancer cell line

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Abracl is a small, highly conserved protein expressed in all eukaryotic organisms except fungi. Previous studies in the Neuro2A cell line have shown that Abracl expression is downregulated upon the induction of differentiation; this observation along with the high expression levels of Abracl detected in the subventricular zone of the subpallium at the peak of embryonic neurogenesis, suggest that it may be involved in proliferation and thus probably have a role in cancer. In order to investigate this hypothesis we generated Neuro2A clones overexpressing Abracl and used them to study its oncogenic potential. To this end we performed proliferation and colony formation assays. In addition, we studied the migratory activity using wound healing assays. Our results show that overexpression of Abracl results in an increase of the proliferation rate as well as of the migratory potential of the cells, in line with a potential role in carcinogenesis. We are currently analyzing the effects of the downregulation of Abracl in knock out clones of Neuro2A cells generated by CRISPR/Cas9.

Keywords: Cancer, ABRACL, Proliferation, Migration

P113

Direct reprogramming into the ependymal cell fate is induced by the GemC1 and MclDas proteins

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The ependymal cells of the brain are specialized epithelial cells lining the walls of the lateral ventricles and constitute a crucial component of the neurogenic subventricular zone niche. They bear characteristic hair-like structures, known as cilia, that are responsible for cerebrospinal fluid (CSF) flow throughout the ventricles. Abberations in the generation/differentiation or functionality of ependymal cells have been reported to provoke abnormal CSF accumulation resulting in hydrocephalus.

Our lab has highlighted the importance of two members of the geminin superfamily; GemC1 and MclDas in the ependymal cells differentiation. They are expressed early on during embryogenesis being responsible for the ependymal fate acquisition of neural progenitor cells. GemC1 is the most upstream regulator of this pathway, activating the expression of important modulators of multiciliogenesis, including MclDas. Our research team has developed GemC1-deficient mice, that lack ependymal cells and develop hydrocephalus.

In order to study the reprogramming potential of the above mentioned modulating genes, we induced their ectopic expression in an *ex vivo* culture of cortical astrocytes and mouse embryonic stem cells (mESCs).

Astrocytes are the most abundant astroglial cells in the mammalian brain and are responsible for preserving its structure and homeostasis. They have also been reported to be activated upon hydrocephalus occurrence, making them great potential targets for treating this condition. ESCs are being studied heavily in the fields of regenerative medicine and tissue engineering. Our efforts regarding their reprogramming have been focused on their targeted differentiation towards the ependymal lineage.

Our data shows that GemC1 and MclDas can successfully induce astrocytes and mESCs reprogramming into the ependymal cell lineage. The two factors present substantial differences in their reprogramming capacity, while MclDas is capable of inducing the generation of fully functional ependymal cells. These findings could be of great importance for the development of novel therapies for hydrocephalus treatment.

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P114

The effect of GCN5 in *Arabidopsis thaliana* flower responses to gibberellins. Characterization of the *rga-t2;gcn5-6* double mutant

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Histone acetyltransferases (HAT) can modify the amino-terminal tails of the core histone proteins via acetylation. The GCN5 protein is a HAT that specifically acetylate H3K14 residues. GCN5 has been associated with cell division and differentiation, meristem function, root, stem, foliar and floral development, and responses to environmental conditions. The flowers of *gcn5-6* plants display reduced length of stamen and exhibit male sterility relative to the wild type plants. This effect could be arise from defects on gibberellin signaling. Gibberellins (GAs) are plant hormones essential for stem and root elongation, leaf development, and flower initiation. The signaling pathway of bioactive GAs is based on the proteolysis of their repressors, DELLA proteins, by the 26S proteasome pathway. The *REPRESSOR OF GA (RGA)* gene encodes a DELLA protein. RGA represses plant growth, inflorescence, and flower and seed development. This research aims to study the genetic interaction of RGA and GCN5 genes, during the first flowers of *Arabidopsis thaliana* inflorescence. We observed that the reduced elongation of stamen filament of *gcn5-6* mutants is reversed in the *rga-t2;gcn5-6* double mutant. Furthermore, the expression of the gibberellin biosynthesis gene *GA3ox1*, and the DELLA *GAI* was reduced in *gcn5-6* flowers. We found that both gene expression is reversed in *rga-t2;gcn5-6* flowers correlated with suppression of the stamens filament elongation. These results suggest that GCN5 is a positive regulator of stamens elongation, while RGA could suppress stamens elongation by affecting GCN5 action.

P115

Deciphering the role of ciliary protein AHI1 in cortical development

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The cerebral cortex is one of the most complex structures, and malfunctions during its development lead to severe brain disorders such as the malformations of cortical development (MCDs) in humans. Preliminary studies show that disruption of the primary cilium, a small organelle serving as a cellular antenna, can lead to MCDs suggesting its potential role in cortical development. While primary cilia in mice regulate the cell cycle of neural progenitor cells (NPCs) and affect neuronal migration, their role in human brain development is vague. Interestingly, mutations in the ciliary-associated gene *AHI1* have been identified in the MCD polymicrogyria. Thus, our aim is to investigate the role of primary cilia, using *AHI1* as a candidate gene, during cortical development and scrutinize the mechanisms which upon disruption might lead to MCDs. By comparing single-cell RNA sequencing datasets, we first estimated any potential species-specific and cell-type-specific *AHI1* expression. We have shown that *AHI1* is higher expressed in NPCs than neurons in humans, while the opposite pattern is observed in all other animal models tested. To dissect its role in vivo, we have provoked ectopic *AHI1* overexpression or silencing in the developing mouse cortex. We examined the number and distribution of apical and basal progenitors as well as the neuronal output upon *Ahi1* manipulation. Our data suggest that *AHI1* manipulation disrupts the number and distribution of NPCs and neurons in the developing cortex. Hence, we sought to investigate potential deficits in their proliferation and differentiation capacity. To delve into the human-specific mechanisms that underlie cortical development, we aim to perform *AHI1* manipulation in human brain organoids which will be used as human-specific models for MCDs. The results of this project will give insight into the role of primary cilia in human cortical development, as well as the key mechanisms regulating MCDs.

P116

Unraveling the functional role of aldehyde dehydrogenase 3A1 (ALDH3A1) in human corneal epithelium

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Cornea endures constant stress serving as protective barrier against environmental insults. Many enzymatic and non-enzymatic antioxidants are present in the corneal tissue orchestrating a robust antioxidant defense system. ALDH3A1 functions in the corneal epithelium as a crystallin implying an integral role in tissue's functionality and structure. Its specific functions in the cornea range from metabolic to non-metabolic, including homeostatic roles in cellular proliferation and differentiation. Here, we investigated the molecular mechanisms underlying the cytoprotective role of ALDH3A1 in human corneal epithelial cells (HCE-2) under normal, oxidative and genotoxic conditions. Using an isogenic HCE-2 cell line differing in the expression of ALDH3A1, we assessed a differentiated cytoprotective behavior in association with ALDH3A1 expression. ALDH3A1 exhibited a protective role in HCE-2 cells by hindering the H₂O₂- or etoposide-induced apoptosis, accompanied by lower levels of the DNA damage marker, γH2Ax. Evaluation of the DNA damage response protein p53 showed a significant increase in the protein levels as well as in the phosphorylation of p53 at Ser15 in HCE-2/ALDH3A1 cells under treatment conditions compared to control. Interestingly, although ALDH3A1 is a cytoplasmic enzyme, it is also found in the nucleus of corneal epithelial cells. In association with previous reports implying potential nuclear roles, we studied the localization of ALDH3A1 under both normal and stress conditions, however no further enhancement of ALDH3A1 nuclear localization was observed neither under oxidative nor genotoxic conditions. We further investigated the potential mechanism of ALDH3A1 nuclear transportation by performing site-directed mutagenesis on a previously reported potential NLS signal at residues 265-281 of the protein's amino sequence, however the results indicated that the particular site is not associated with the translocation mechanism. We conclude that the clarification of the exact implication of ALDH3A1 in corneal homeostasis could be valuable for exploring novel therapeutic strategies, aiming at various corneal pathologies.

The study has been conducted by the project "InTechThrace: Integrated Technologies in biomedical research: multilevel biomarker analysis in Thrace" (MIS Code 5047285), under the Operational Program "Competitiveness, Entrepreneurship & Innovation" (EPAnEK), co-funded by the European Regional Development Fund (ERDF) and national resources (Partnership Agreement 2014-2020).

P117

In search of the molecular basis of Sec24 interaction with cargoes sorted in the plasma membrane via Golgi-bypass

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In the early secretory pathway, the exit of newly synthesized membrane proteins from the endoplasmic reticulum (ER) takes place at specialized regions called ER exit sites, where cargoes are collected and pack into COPII secretory vesicles, which are destined to fuse with the cis-Golgi. Following Golgi maturation, membrane proteins exit from the trans-Golgi network in clathrin coated vesicles directed to the PM¹. However, our recent findings in *Aspergillus nidulans* showed that several transmembrane cargoes follow an 'unconventional' sorting pathway that bypasses the Golgi^{2,3}, which in turn suggests the existence of distinct cargo-specific COPII subpopulations. Selective recruitment of membrane cargoes in COPII by Sec24 is mediated by direct interactions between cargo-binding sites in Sec24 and specific sorting motifs in cargoes^{4,5}. Here we investigate the molecular basis of Sec24 interactions with the UapA transporter, a paradigmatic Golgi bypasser. For this, we develop a controllable system to repress the synthesis of the endogenous Sec24 and use it to investigate the functional effect of systematically designed Sec24 mutations carried in a plasmid vector introduced by reverse genetics. In parallel, we re-investigate the role of a cytoplasmically located N-terminal motif of UapA in ER-exit and PM localization. Our results show that i) amino acid substitutions in conserved or semi-conserved residues located in the A and B cargo-binding sites of Sec24 are critical for UapA ER-exit and/or fungal growth ii) a Tyr residue in the N-terminal motif of UapA is essential for ER-exit and seemingly crucial for recognition by Sec24.

¹ Gomez-Navarro, N. & Miller, E. J. *Cell Biol.* 215, 769–778 (2016)

² Dimou, S. et al. *EMBO Rep.* 21, (2020)

³ Dimou S, et al. *Front Cell Dev Biol.* 10:852028 (2022)

⁴ Chatterjee, S., et al. *Traffic* (2021)

⁵ Miller, E. A. et al. *Cell* 114, 497–509 (2003)

P118

Effect of cannabidiol on behavioral parameters and acetylcholinesterase activity through ageing

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Age-related changes have been associated with behavioral manifestations and cholinergic system alternations. Due to its safety profile and lack of psychotropic action, cannabidiol (CBD) is a cannabinoid on interest, with many reports of pharmacological effects in various pathological models, ranging from inflammatory and neurodegenerative diseases to polycystic arthritis, epilepsy and autoimmune diseases etc. The aims of the present study were to investigate the effect of cannabidiol treatment on anxiety-like behavior, mobility and on acetylcholinesterase (AChE) activity in specific brain regions (cerebral cortex, striatum, hippocampus and cerebellum) of male mice. The mice were divided into 3 age groups: a) adolescent [1-month-old (m.o.)], b) adult (3-4 m.o.), and c) aged (13-14 m.o.). Each age group was divided in 2 groups: CBD group (CBD 10mg/kg, 10%DMSO, 2% tween-80) and Control group (saline, 10%DMSO, 2% tween-80). The CBD was administrated for 10 days and 24h after the last administration the behavioral analysis has been performed. Anxiety-like behavior and mobility were assessed by using the open field test in a 10min task. The AChE activity was determined in both salt-soluble (SS-AChE) and detergent-soluble (DS-AChE) fraction in the 4 brain regions, by using Ellman's colorimetric method. Behavioral studies revealed that the adolescent and aged group appeared to be highly anxious in comparison to adult group. Moreover, the results showed an anxiolytic-like activity and an increase of mobility after the CBD treatment. The results exhibited both SS- and DS-AChE activity in adolescent and aged group to be significantly lower than the adult group, in all brain regions studied. Furthermore, CBD treatment reduced acetylcholinesterase activity of both fractions in all age group.

P119

Cross-talk between the cellular response to hypoxia and the Integrated Stress Response (ISR) in cancer cells

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Cancer cells in solid tumors, especially under cytotoxic treatment, often encounter stress conditions (oxidative, osmotic or ER stress, nutrient depletion and UV radiation). As a defense mechanism, cells respond by activation of the integrated stress response (ISR), characterized by phosphorylation of the eukaryotic translation initiation factor eIF2 α and inhibition of global protein translation. Under ISR, stalled mRNAs undergo condensation and accumulation in cytoplasmic stress granules (SGs). In addition to ISR-inducing stress, cancer cells are also often exposed to low oxygen (hypoxia) due to the poor or aberrant vascularization of solid tumors. The adaptation to hypoxic conditions is mainly mediated by the Hypoxia Inducible Factors (HIFs). Both ISR and the response to hypoxia, facilitate cancer cell survival and resistance to chemotherapy. To study the effects of hypoxia on ISR and, in particular, SG formation, different cancer cell lines (HeLa, Huh7, MCF7, A459 and H1299) were incubated under 21% O₂ (normoxia) or 1% O₂ (hypoxia) in the absence or presence of arsenite. SG formation was monitored by immunofluorescence microscopy using an antibody against the SG protein G3BP1. Arsenite treatment triggered ISR, as documented by increased levels of phospho-eIF2 α and SG-formation, both under normoxia and hypoxia. Moreover, arsenite down-regulated the expression of HIF-1 α under hypoxia. On the other hand, hypoxia itself did not cause phosphorylation of eIF2 α or SG formation, but affected the average size and/or number of SGs formed per cell under arsenite treatment in all cell lines. Furthermore, hypoxia prevented the formation of SGs in a significant percentage of A549 lung adenocarcinoma cells treated with arsenite, suggesting the involvement of the NRF2 pathway, which is constitutively active in these cells. These results suggest a cross-talk between ISR and the response to hypoxia, the understanding of which may allow simultaneous targeting of multiple survival mechanisms as an efficient form of cancer treatment.

P120

Novel components of cell-matrix adhesions revealed by proteomic analysis

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Cell adhesion to extracellular matrix (ECM) is central to all essential cellular functions, including cell-migration and signalling. Cell-matrix adhesion is mediated by a dynamic network of cytoskeletal and signalling proteins organised around integrin transmembrane receptors, called adhesome. The temporal and spatial composition of adhesome defines the strength of adhesion and regulates cellular homeostasis. Previous studies elucidating the adhesome composition of fibroblasts and cancer cells have shed light into the molecular mechanisms of tissue homeostasis and cancer. Here, we set our to decipher the molecular architecture of adhesome in endothelial cells (EC) and gain insight into the role of cell-matrix adhesions in blood vessels. For this, we performed proteomic analysis of isolated cell-matrix adhesions from primary mouse ECs. Specifically, we investigated changes in the protein composition of endothelial adhesome upon deletion of Talin, a key cell-matrix adhesion protein. Our analysis revealed the essential components of endothelial adhesions and uncovered novel members of integrin adhesome. We discovered Cytoplasmic activation and proliferation-associated protein- 1 (Caprin-1) to be a new adhesome component with a talin-dependent localisation at cell-matrix adhesions. Collectively, our findings highlight a versatile composition of endothelial cell-matrix adhesions with novel components and distinct functions.

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P121

MclDas cell cycle regulation is necessary for genome integrity

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Maintaining the balance between proliferation and differentiation is crucial for multicellular organisms and this is achieved through the orchestration of factors that control these processes. Geminin superfamily members -Geminin, MclDas and GemC1- is a group of distantly related coiled-coil proteins characterized for their roles both in DNA replication initiation and multiciliated cell differentiation¹. Here, we describe how MclDas is regulated during the cell cycle and the impact of its deregulation on genome integrity. The levels of MclDas drop after anaphase and increase again before S phase. MclDas is an APC/C substrate, and it is recognized through two destruction sequences, DBox and ABBA motifs. A mutational analysis on MclDas destruction sequences was performed and we showed that the cell cycle profile of the cells was unaffected. However, the non-degradable forms of MclDas led to genomic instability phenotypes, such as DNA bridges, micronuclei, multinuclear cells and 53BP1 nuclear bodies. These phenotypes are linked to DNA replication perturbation, illustrating an important role for MclDas in genome duplication²we provide an overview of how proliferating eukaryotic cells overcome one of the main threats to genome stability: incomplete genomic DNA replication during S phase. We discuss why it is currently accepted that double fork stalling (DFS). The members of the Geminin superfamily are involved in an increased number of cellular processes, therefore understanding their functions will allow their role in the balance between proliferation and differentiation to be elucidated.

¹ Arbi, M., Pefani, D.-E., Taraviras, S. & Lygerou, Z. Controlling centriole numbers: Geminin family members as master regulators of centriole amplification and multiciliogenesis. *Chromosoma* 127, 151–174 (2018).

² Bertolin, A. P., Hoffmann, J. S. & Gottifredi, V. Under-replicated DNA: The byproduct of large genomes? *Cancers* 12, 1–20 (2020).

P122

Effects of Cannabidiol pre-treatment on behavioral, inflammatory and biochemical markers on male mice after Concanavalin-A intoxication

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Cannabidiol (CBD) is the major non-psychotropic phytocannabinoid derived from the plant *Cannabis sativa*. CBD is a pharmacologically broad-spectrum drug that has many beneficial pharmacological effects, including anti-inflammatory and antioxidant effects. In recent years CBD has an increased interest as a treatment for a range of neuropsychiatric disorders. Concanavalin-A (Con-A) is a lectin from the jack bean, *Canavalia ensiformis*, has several adverse effects including cytotoxicity, apoptosis, and inflammation. The aim of present study was to investigate the effect of cannabidiol on a) anxiety-like behavior b) inflammatory markers in plasma c) acetylcholinesterase (AChE) isoforms (G1, G4) activity in specific brain regions (cortex, striatum and diencephalon) of adult male mice after Con-A intoxication. Mice were pre-treated orally with CBD (20 mg/kg, gavage) for five days, and challenged with saline or Con-A (20mg/kg; i.v.) on the fifth day. The behavioral analysis was assessed by using the open-field test in order to evaluate the anxiety-like behavior 1 hour after the last administration. The inflammatory analysis was assessed by determining the IL-2, IL-4 and INF- γ levels on plasma by enzyme-linked immunosorbent assay. The activity of G1 and G4 AChE's isoforms was determined, by using Ellman's colorimetric method. Behavioral studies revealed an anxiolytic-like behavior after the CBD treatment. Con-A intoxication has been found to increase anxiety-like behavior. Moreover, the mice group that has been treated with CBD and Con-A has shown anxiolytic-like behavior compared to the Con-A administered group. The results reveal that IL-2, IL-4 and INF- γ levels on plasma were increased after Con-A intoxication (inflammation index) and were reduced when mice were pre-treated with CBD. The results indicate a decrease in AChE activity in the cerebral cortex, striatum and diencephalon in both G1 and G4 isoforms in the CBD group but also in the Con-A group as well as in the co-administration of Con-A and CBD.

P123

Expression of metalloproteases in breast cancer cells in the presence of statins

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Breast cancer is the most common type of cancer that affects women with the incidence rate reaching 30% and even exceeding in number lung cancer cases. The disease is characterized as multifactorial because it is triggered by a combination of genetic and environmental factors. Existing therapies are aiming to remove cancerous cells that are found in the mass tissue or even in adjacent tissues, such as lymph nodes. The uncontrolled cell proliferation is caused by proteins (e.g. cytokines), chemical substances (e.g. hormones) and various enzymes like metalloproteases, enzymes that degrade the extracellular space and therefore promoting cancer progression. In this study, metalloprotease expression is studied in two breast cancer cell lines, under the presence of simvastatin, drug belonging to statins, chemical substances used for lowering blood cholesterol levels. These substances block HMG-CoA reductase, and thus inhibit the first step of sterol biosynthesis. Two cell lines are used, one of hormone-dependent breast cancer, MCF-7 and the other of triple negative breast cancer, MDA-MB-231.

The techniques that are carried out in the present work, included basic experiments in cell cultures, gene expression at mRNA levels, and detection of enzymatic activity by gelatin zymography. The results obtained suggested that simvastatin affected metalloproteases and in particular reduced their expression, in many cases in a dose-dependent manner. Since metalloproteases contribute to key points in cancer development, such as angiogenesis and metastasis, the results of the present study suggest that simvastatin could be used in chemotherapy regimens to treat breast cancer patients.

P124

Identification of arbuscular mycorrhizal fungi in soil using Ion Torrent Technology: A comparative evaluation of primer sets targeting rRNA gene cluster.

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The study of rhizosphere microbiome, in particular of important symbiotic microorganisms such as arbuscular mycorrhizal fungi (AMF), is of key importance for unraveling its interaction mechanisms with plants. Such knowledge is potentially valuable for agriculture and industrial applications. Through amplicon-based next-generation sequencing, rDNA gene cluster can be used to identify and discriminate AMF species in complex environmental samples. There are numerous nuclear rDNA targeting primers that can be used to study AMF species, but the optimal selection of these primers remains a controversial issue. Here, we aim to decipher the ability of five primer sets to accurately describe an AMF reference community. Exploiting the Ion Torrent technology, we compare five specific primer sets, corresponding to the V4 of the 18S region, the D2 domain of the 28S region or the ITS2 region, using a mock community of ten common AMF species. Our results revealed that the 18S-primer sets allowed for accurate identification of most of the species presented, while ITS2-primer sets and the LSU-primer set could infer an adequate diversity of AMF but obtain only a few of the species presented. We demonstrated that the selection of primers and the rRNA gene database are two of the most critical factors affecting AMF taxonomy. The preservation of plant diversity entails an in-depth understanding of belowground biodiversity focusing on the symbiotic components, such as AMF. Thus, the collection of further research data is crucial to promoting the development of straight-forward protocols in sequencing-based microbial ecology studies.

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Genomic and phenotypic evaluation of the antimicrobial potential of *Lacticaseibacillus paracasei* SP5 against common human enteropathogens

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Lactobacilli are avid producers of antimicrobial compounds responsible for their adaptation and survival in microbe-rich matrixes. The bactericidal or bacteriostatic ability of strains could be further exploited in the food or biomedical industry to produce foodstuffs and supplements with elevated properties. *Lacticaseibacillus paracasei* SP5 is a newly sequenced strain, previously shown to possess desirable probiotic properties, including antiproliferative and adhesion capacity, as well as biotechnological potential. In this study, comprehensive bioinformatic analysis and experimental validation was performed to examine the antimicrobial capacity of *Lc. paracasei* SP5. More specifically, annotation of bacteriocin clusters was performed with BAGEL4, also utilizing the Kyoto Encyclopedia of Genes and Genomes (KEGG) and Clusters of Orthologous genes (COGs) databases. It was found that the strain carries 5 distinct clusters coding for 3 core peptides: N-acetylmuramoyl-L-alanine amidase and two class IIb bacteriocins. Additionally, pathways and genes involved in the production of other antimicrobial compounds including lactic acid, ethanol, hydrogen peroxide and reactive oxygen species were identified. The antimicrobial properties of the strain were validated in vitro against the clinically relevant enteropathogens *Staphylococcus aureus*, *Salmonella enterica* ser. Enteritidis and *Escherichia coli*. More specifically, *Lc. paracasei* SP5 effectively co-aggregated with all three pathogens and limited attachment onto HT-29 cell monolayers after 4 h of co-incubation. Furthermore, cell-free culture supernatants (CFCS) limited viability and biofilm formation capacity of the pathogens with variable efficiency, as evidenced using a microbiological assay and confocal microscopy. Future studies will focus on the characterization of the structure and function of metabolites produced by *Lc. paracasei* SP5 mediating this phenotype.

P126

Chemical profile, antioxidant properties and antiproliferative potential of ethanolic *Haberlea rhodopensis* extracts

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Haberlea rhodopensis is a Balcan endemic plant that is known for its resilience to drought and other stress conditions. It belongs to the Gesneriaceae family, which includes plants with medicinal and health promoting properties. The aim of the study was (i) to characterize the chemical profile of *H. rhodopensis* ethanolic extract (HEE) prepared from the leaves of the plants, (ii) to study the antioxidant properties of HEE and (iii) to investigate the antiproliferative potential of HEE against a panel of preclinical cancer models. UPLC-MS/MS analysis identified that the HEE contains high levels of both flavonoids and phenolic compounds, low levels of monoterpenoids and condensed tannins, high levels of soluble protein and pigments (a-/b-chlorophyll, b-lycopene and carotenoids) and low levels of soluble sugars. The extract demonstrated remarkable antioxidant activity as it was evaluated by the 2,2-di-phenyl-1-picrylhydrazyl (DPPH) and 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays. The antioxidant potential of HEE was further validated against H₂O₂-induced oxidative damage using human keratinocytes (HaCaT). HaCaT cells pre-incubated with non-toxic concentration of HEE (20µg/ml) for 24 h resulted in increased cell viability following exposure to H₂O₂. The effect of HEE on the expression profile of a panel of target-genes associated with the antioxidant pathway NFK2/KEAP1 is currently being investigated. Finally, the antiproliferative activity of the extract was assessed against six human cancer cell lines by employing the sulforhodamine-B (SRB) assay. Hepatoma (HepG3) and non-small cell lung adenocarcinoma (A459) cell lines were amongst the most sensitive to the antiproliferative effects of HEE. In conclusion, the ethanolic extract of *H. rhodopensis* exerts promising biological properties that seek further investigation providing promising potential as natural source of antioxidant and anticancer candidates for future exploitation in biomedical applications.

P127

Expression and purification of human Interferon alpha 2a in the methylotrophic yeast *Pichia pastoris*

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Interferon alpha 2a (IFN α 2a) belongs to the family of type I interferons, critical cytokines for the immune system regulation. In humans, IFN α 2 is used extensively as a therapeutic agent against AIDS, Hepatitis B and C and several types of malignancies. The aim of this study was to express IFN α 2a in the methylotrophic yeast *Pichia pastoris*. *Pichia pastoris* offers easy, rapid and low-cost culture, straightforward genetic manipulation protocols and has some of the advantages of higher expression systems, such as protein folding and availability of post translational modifications, including glycosylation. In our study, the gene encoding the human mature IFN α 2a was cloned in pPink α HC vector with the removable tags TAP-tag and His-tag. After induction of expression, the secreted tagged IFN α 2a was purified with affinity chromatography. The human mature IFN α 2a, liberated from tags, was collected after treatment with TEV protease, followed by a second cycle of affinity chromatography. The biological activity of the recombinant IFN α 2a is being assessed and compared to that of IFN α 2a prescribed as medication. Real-time PCR revealed that IFN α 2a produced by *Pichia pastoris*, induced the up-regulation of various interferon stimulated genes (ISGs) such as MX1, OAS1, IFIT1, IFIT3 and TRAIL in A549 cells. Overall, our results indicate that the eukaryotic production of IFN α 2a could be potentially used for the production of polypeptides with improved structural stability and biological activity.

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P128

Collagen-containing fish sidestream-derived protein hydrolysates support skin repair via chemokine induction

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Wound healing is a dynamic and complex process involving four interconnected stages, in which chemokines are key orchestrators. Nutrition is an important factor that directly affects skin homeostasis, whereas malnutrition impairs tissue healing. Every year, a large number of sidestreams are produced by the fish processing industry, which are either discarded or underutilized. This raw material, which contains a great amount of muscle and connective tissue with high protein content, can be subjected to enzymatic hydrolysis to generate protein hydrolysates, a source of bioactive peptides. In addition to their biological activity, many of these marine derivatives also have great nutritional value; therefore, a beneficial effect on human health could emerge.

In the present study, we used fish sidestream-derived protein hydrolysates including fish collagen as dietary supplements to test their impact on the skin repair process using an in vivo cutaneous wound healing model. We explored potential differences in wound closure and histological morphology between the diet groups and analyzed the expression and production of factors that participate in different stages of the repair process.

Dietary supplementation with fish sidestream-derived collagen alone (Collagen), or in combination with a protein hydrolysate derived from salmon heads (HSH), resulted in accelerated healing. Tissues from mice fed with collagen-containing supplements exhibited an increase in the expression levels of chemokines, important for the recruitment of immune cells into the damaged wound region. According to the chemical analysis, Collagen had the highest protein content and HSH contained a large amount of zinc, which is known to support immune responses. Our findings suggest that a 5%-supplemented diet with marine collagen-enriched supplements promotes tissue repair in the model of cutaneous wound healing, proposing a novel health-promoting use of fish sidestreams.

P129

Evaluating the effect of probiotics in the gut microbial community of healthy mice

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Intestinal microbiome holds a key-role in a variety of biological functions; dysbiosis has been associated with a number of human diseases. Several studies point to the beneficial effects of probiotic administration for the management of illnesses; little is known, however, about their effects in healthy individuals. In this study, we aim to analyze the structure of the gut microbial community of healthy mice following probiotic administration, as well as to investigate whether these changes may have a role in preserving health. To this end, we have analyzed the gut microbial community following a 6-week intervention with a novel probiotic strain of *Lactiplantibacillus pentosus*. We show that *L. pentosus* administration in healthy mice resulted in a significant increase of the rate of *Firmicutes/Bacteroidetes* indicating that this intervention promotes intestinal homeostasis; *Clostridia* and *Lachnospiraceae* were the taxa more affected. Our results, though preliminary, suggest that prolonged probiotic administration has beneficial effects; we are currently analyzing the tissues, the blood as well as the contents of different areas of the gut to confirm whether such interventions promote health.

P130

A method for the purification of analytical grade phycocyanin from *Arthrospira platensis* for biotechnological purposes

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Phycocyanin (C-PC) is a protein found in cyanobacteria, such as *Arthrospira platensis*, also known as Spirulina. C-PC is a hydrophilic fluorescent molecule with a natural bright blue color and exhibits anti-oxidant and anti-inflammatory properties. C-PC belongs to the family of phycobiliproteins and along with allophycocyanin (APC) and phycoerythrin (PE) composes larger protein complexes called phycobilisomes (PBS), which play an important role for bacteria survival in different environments. These proteins harvest solar energy from visible spectrum in a long range of 450 to 670 nm and relay photons to the photosynthetic center. C-PC exhibits a large stoke's shift as it absorbs light at 620nm and emits at 642nm. Currently, it is widely used in the food and cosmetics industries as a food-grade, bio-compatible, hypoallergic colorant, in various biotechnological applications as a fluorophore and in health applications as a label in FACS analysis or as a medical device in microsurgery. In our study we extracted C-PC from fresh *Arthrospira platensis* biomass via mild homogenization of cells. Then, we purified C-PC to analytical grade exploiting a combination of differential ammonium sulfate precipitation steps and ion-exchange chromatography techniques. We have evaluated the quality of the produced C-PC, its stability and solubility and we are developing methodologies for linking C-PC to certain biomolecules in order to use it as a fluorescent probe. Our study highlights the usefulness of C-PC for biotechnological applications.

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P131

Evaluation of the environmental DNA (eDNA) method to estimate the distribution of the critically endangered charophyte *Chara hispida f. corfuensis* in Kaiafas lagoon

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Freshwater biodiversity assessment using an innovative eDNA method provides a new powerful tool for water quality research. Recently, the innovative method of eDNA for the direct detection of species-specific DNA from water, has been applied in many monitoring surveys of aquatic species, throughout the world. Aquatic macrophytes are widely used as indicators of water quality and ecological integrity in all types of freshwater ecosystems (lakes, rivers, lagoons). *Chara hispida f. corfuensis* is a submerged plant of high conservation importance as it is classified as critically endangered according to IUCN and is included in the Red List of Charophytes. It used to form extensive and dense beds in the lagoon of Kaiafas, but continuous nutrient discharges from agricultures and other anthropogenic pressures have diminished its population. Here, we present a method based on eDNA to biomonitor this important species without disturbing its' natural habitat, as it often happens during conventional survey methods. Water sample was collected from the surface of Kaiafas as also plant tissue from aquatic macrophytes *Chara hispida f. corfuensis*, *Najas marina* and *Potamogeton pectinatus* (sympatric species). Water samples were filtrated through cellulose nitrate (CN) filter membranes in order to capture eDNA. Then, eDNA was used as a template in PCR combining not only species- specific primers that amplifies the barcode- gene (matK) of *Chara hispida f. corfuensis*, but also universal primers that amplifies a more conservative region of gene matK. Results showed positive amplification only with universal primers, indicated that *Chara hispida f. corfuensis* is not present in the particular habitat of the water sampling station.

P132

A primary investigation of the microbial ecosystem of the Greek PDO cheese Sfela, Sfela touloumotiri and Xerosfeli

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The objective of the present study was the characterization of the microbiome of the traditional Greek PDO Sfela cheese. Sfela is a white brined cheese, characterized by high salinity and is produced from ovine or caprine milk in the regions of Messinia and Laconia. During this study, two samples of Sfela cheeses were selected as well as Sfela Touloumotiri and Xerosfeli, which are close variants of the traditional cheese. The techniques selected for the identification of the microflora were 16S rDNA amplicon sequencing and shotgun metagenomics analysis. The first technique allowed the characterization of the populations at genus level. More specifically, it appears that in the samples of cheese some of the most abundant genera were *Lactobacillus*, *Streptococcus* and *Lactococcus*. The shotgun metagenomics analysis revealed the bacterial and yeasts species forming the microbial ecosystem of the studied cheeses. In the two Sfela samples, the species found in large populations were *Streptococcus thermophilus*, *Lactococcus lactis*, *Levilactobacillus brevis*, *Latilactobacillus curvatus*, *Lactobacillus delbrueckii* etc. In Sfela touloumotiri, the most abundant species were *Tetragenococcus halophilus* and *Lactococcus lactis* as well as the yeast *Debaryomyces hansenii*. In Xerosfeli, the two predominant species of bacteria were *Streptococcus thermophilus* and *Lactobacillus delbrueckii*. Moreover, metagenome-assembled genomes (MAGs) were used to determine the functional properties of the different cheese microbiomes. These findings may allow us to search and isolate targeted strains of bacteria and yeasts with desirable technological properties to be used in the production of Sfela cheese.

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P133

Molecular and biochemical properties of a methylesterase (OeEAME) which converts oleuropein aglycone to oleacein in *Olea europaea*

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In the last decades, researchers from diverse fields have focused on the secondary metabolism of plants in order to elucidate the biosynthetic machinery which leads to the production of certain metabolites, often known for their therapeutic properties. Olive trees biosynthesize an uncommon type of secoiridoids, known as oleosides, which are only found in members of the Oleaceae family. Oleosides are found in olive tree and in extra virgin olive oil (EVOO) and have gained significant interest because of their beneficial effect on human health. Oleuropein is the dominant oleoside which is also involved in a mighty defense mechanism of olive tree engaging also the oleuropein β -glucosidase (OeGLU) which detonate oleuropein to form the bioactive aglycone form. Here we shed light in the molecular and biochemical properties of an already characterized methylesterase (OeEAME) which converts oleuropein aglycone to oleacein. Oleacein can be found in EVOO and is known to contribute in the beneficial aspects of this vegetative oil for human health. We investigated the transcriptional profile of these enzymes in various tissues, the subcellular localization *in planta* and explored the quaternary structure of OeEAME's. Both enzymes had a comparable transcriptional pattern among the analyzed plant tissues (leaves, flowers, buds, stems and roots). To investigate the subcellular localization of OeEAME, N- or C-terminal YFP-fused constructs of OeEAME were generated and revealed that this enzyme exhibits a nucleocytoplasmic localization. Bimolecular fluorescent complementation (BiFC) analysis suggested that OeEAME homodimerizes and further split-YFP analyses revealed that OeEAME is able to heterodimerize with OeGLU suggesting that both enzymes orchestrate a heteromeric complex. These results expands our understanding on two enzymes that are directly shaping the organoleptic properties of EVOOs by performing sequential enzymatic reactions *in planta*.

P134

Investigation of novel biomarkers for monitoring intestinal health in broilers challenged by *Eimeria* spp

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In intensively reared animals, such as broiler chickens, intestinal health is crucial for feed efficiency and growth. Finding trustworthy, highly accurate biomarkers to assess intestinal inflammation and barrier function may be a promising first step toward the early recognition and management of economically important diseases in poultry. The objective of this study was to evaluate intestinal health biomarkers in the serum, faeces and tissues of broiler chicks that were experimentally challenged by *Eimeria* spp. A total of 32 one-day-old broiler chicks (Ross® 308) were randomly allocated into two treatment groups, with 4 replicates, according to the following experimental design: **Group A:** the negative control and **Group B:** birds of which were challenged at the 14th day of age with an inoculum-blend of *E. acervulina*, *E. maxima* and *E. tenella*. Seven days post-infection, serum Interleukin-10 (IL-10) and faecal ovotransferrin concentration were measured by sandwich and competitive ELISA, respectively, while the gene expression levels of tight junction protein “occludin” in jejunal segments were quantified using quantitative Real-Time PCR. The statistical analysis and the evaluation of the experimental data revealed that the challenge with *Eimeria* spp. reduced significantly ($P \leq 0.05$) the expression of occludin, as well as the faecal concentration of ovotransferrin, whereas the levels of IL-10 in the serum were not significantly ($P > 0.05$) affected. This study indicates that the expression of tight junction protein occludin and the faecal concentration of ovotransferrin could be promising biomarkers for the evaluation of gut health in broilers challenged by *Eimeria* spp. However, validation will be a major challenge, due to the complexity of the factors that affect intestinal health in the field.

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In silico investigation of potential immunogenic sites in the human recombinant polypeptides Interleukin-2, Erythropoietin and Interferon alpha 2a

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The recombinant pharmaceutical protein industry has evolved rapidly since the production of human insulin in 1982. Today there are more than 300 biopharmaceutical products on the market, including therapeutic proteins, with sales exceeding 100 billion US\$ annually, produced by a variety of expression systems and approved by the FDA. In our research we selected to study 3 major pharmaceutical proteins: Interleukin-2 (IL-2), Erythropoietin (Epo) and Interferon alpha 2a (IFN α 2a). Specifically, in cancer immunotherapy clinical approaches IL-2 is used as an anticancer agent to treat melanoma and metastatic kidney cancer, while Epo is administered to patients with severe chronic kidney diseases, HIV and cancer patients receiving chemotherapy or to treat anemia. IFN α 2a has been approved for the treatment of hairy cell leukemia, chronic hepatitis B and C virus infection (HBV & HCV), chronic myeloid leukemia (CML), renal cell carcinoma, Kaposi's sarcoma, T-cell lymphoma, multiple myeloma and condyloma acuminata. Currently, the three polypeptides are developed as recombinant proteins in bacterial expression systems. In order to express these polypeptides in a prototype methylotrophic yeast expression system, we investigated the potential immunogenicity of certain sites along their amino acid sequences. We performed an *in silico* analysis of databases, such as the Immune Epitope Database (IEDB) and SYFPEITHI. The findings of the *in silico* studies were experimentally confirmed by immunoassays, such as enzyme-linked immunosorbent assay (ELISA). Our ultimate goal was to identify potential immunogenic epitopes that, if modified, would reduce the risk of triggering immune responses in patients with chronic conditions that require repeated administration of such drugs.

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P136

An insight into the microbiome of Greek table olives coming from different cultivars at retail

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Even though the evolution of the microbiome of table olives during fermentation process has long been investigated, there is little, or no information regarding the microbial diversity at retail. Thus, the aim of the study was to assess different table olive cultivars for serving as a pool of new starters or adjunct cultures for the production of Greek-style olives. In total, nine olive samples from cv Kalamata, cv Konservolia, and Halkidiki were collected from large supermarkets. Respectively, commercially produced brines were analysed microbiologically (Lactic acid bacteria, enterococci leuconostocs, yeasts) and physicochemically (pH, acidity, %NaCl). Then, total DNA was extracted and subjected to Next Generation Sequencing for the exploration of bacteria and yeasts and molds community. The 16S and ITS rDNA amplicon sequencing analysis allowed the identification of bacterial populations at the genus level. Although differences were observed between the microbial abundance of the samples, the predominant genera were lactic acid bacteria and/or yeasts. Furthermore, the microbiota of the samples was identified at the species level by shotgun metagenomics. The microbial contribution to the technological output of the final product was investigated in cells harvested from simulated olive broth. The results were comparable to the cultivable community identified by MALDI-ToF/ToF. The findings of the study showed that the commercially available table olives could contribute to the collection of microbial isolates with promising properties. In all cases, results should be validated with controlled fermentations.

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Expression and purification of human Interleukin-2 in the methylotrophic yeast *Pichia pastoris*

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Interleukin-2 (IL-2) is a cytokine produced primarily by activated CD4⁺ T cells and is critical for the regulation of the immune system. In humans, IL-2 is administered to enhance T cell immunity in patients with cancer or AIDS and to block binding of Abs to the IL-2 receptor, inhibiting T cell responses in many cases of transplanted tissues. Currently, pharmaceutical human IL-2 is developed as recombinant protein in bacterial expression systems and is available as injectable lyophilized powder. In contrast to current practices, we took advantage of the methylotrophic yeast *Pichia pastoris* in order to express human IL-2 in a secreted form. As a eukaryote, *Pichia pastoris* combines all the advantages of higher expression systems, such as protein folding and availability of post translational modifications, such as glycosylation, with the advantages of conventional bacterial expression systems offering easy, rapid and low-cost culture. Therefore, we cloned a synthetic gene encoding the human mature IL-2 using the *P. pastoris* codon bias in the pPinkAHC vector with the removable affinity tags TAP-tag and His-tag. The purification of the secreted tagged IL-2 was conducted with affinity chromatography, using Ni-NTA agarose beads. Free from the tags, the human mature IL-2 was collected after treatment with TEV protease, followed by a second cycle of affinity chromatography. The structural stability and oligosaccharide composition of the mature polypeptide is being investigated. Using state-of-the art technology, our polypeptide could be used to develop new pharmaceutical forms in which the bioavailability and pharmacokinetics could be evaluated in vivo in mice, aiming to form a polypeptide biosimilar drug with superior characteristics.

Acknowledgement: This research has been co-financed by the European Regional Development Fund of the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH – CREATE – INNOVATE (project code: T2EΔK-00996).



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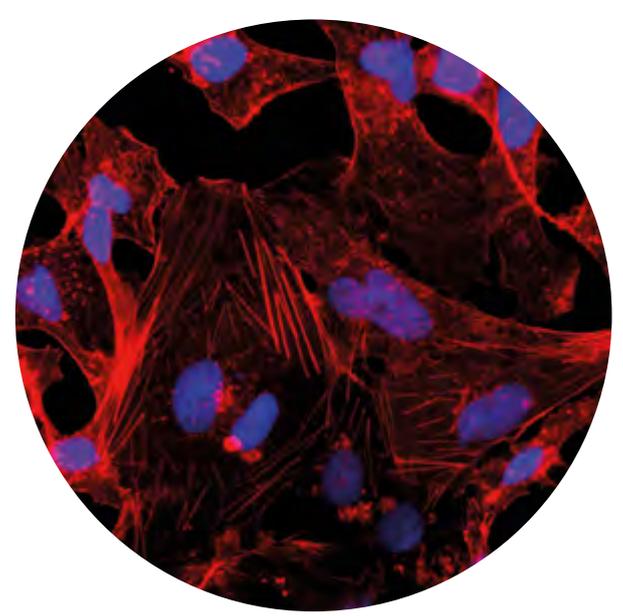
POSTER SESSION 3

SYSTEMS BIOLOGY & BIOINFORMATICS

CHEMICAL BIOLOGY

REGULATION OF GENE EXPRESSION & EPIGENETICS

STRUCTURE AND FUNCTION OF MACROMOLECULES



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Stress-induced affects DNA repair and promotes invasive breast cancer progression

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Characterizing stress-induced promoter-associated antisense lncRNAs (si-paancRNAs) reveals that they play an essential role in cancer pathways. *KDM7A* divergent transcript (*KDM7A-DT*) is one of the si-paancRNAs, whose expression is to be deregulated in breast cancer (BC). The mechanisms leading to aberrant *KDM7A-DT* transcription, biogenesis, and downstream functions in BC types and subtypes have not been studied. In this study, *KDM7A-DT* overexpression and knockdown experiments were carried out using cell lines, biochemical methods, and profiling assays. Integration of experimental models and massive BRCA patient metadata analyses were performed to investigate the structural, functional, and clinical significance of *KDM7A-DT*. We found that *KDM7A-DT* has complex biogenesis, providing full-length and intermediate-sized lncRNAs with different cytoplasmic and nucleus expression distributions. *KDM7A-DT* overexpression in nonmalignant fibroblast cells upregulates p53, CDKN1A, and γ H2AX signaling, resulting in a prolonged cell growth retardation phenotype. *KDM7A-DT* induction by acute oxidative stress in semi-transformed cells is pro-oncogenic and p53-dependent. Metadata revealed full-length *KDM7A-DT* amplification and overexpression in many cancers. In primary BC, these aberrations were associated with TP53-missense mutations in highly aggressive, invasive, basal-like (BL) ductal carcinoma. *KDM7A-DT* affects DNA repair via the NHEJ pathway, inhibits tumor suppressors involved in the epithelial-to-mesenchymal transition, induces oncogenic metabolic changes and G2/M checkpoint arrest, and correlates with histology, aneuploidy, hypoxia, and expression of BC-associated proteins. Data-driven *KDM7A-DT*-coregulated mRNA and protein network is introduced and characterized in the prognostic and diagnostic contexts. In summary, *KDM7A-DT* is a TP53 mutation-associated and copy number-dependent pro-oncogene si-paancRNA that contributes to BC genome instability, initiation, BL subtype progression, invasiveness, and poor outcomes. The *KDM7A-DT*-coregulated sub-network provides prospective molecular targets for classifying basal-like BC, and discovering novel therapeutic strategies.

Acknowledgment: The study has been supported by the project "InTechThrace: Integrated Technologies in biomedical research: multilevel biomarker analysis in Thrace" (MIS Code 5047285), under the Operational Program "Competitiveness, Entrepreneurship & Innovation" (EPAnEK), co-funded by the European Regional Development Fund (ERDF) and national resources (Partnership Agreement 2014-2020) and also, by the Hellenic Foundation for Research and Innovation (HFRI) under the 3rd Call for HFRI PhD Fellowships (Fellowship Number: 05704)

P139**In silico study of triple negative breast cancer: an approach through System Pharmacology****Christina Kolla, George Gavriilidis, Anastasia Tsigonjidou, Georgios Pampalakis***¹Department of Pharmacy, Thessaloniki, Greece, ²Department of Veterinary, Thessaloniki, Greece*

In the present dissertation, in silico analysis of proteomic data from metastatic breast cancer cells MDA-MB-231 (parental) and genetically modified variants carrying the expression cDNA encoding kallikrein 6 (KLK6) (C5-high expression, KLK6 C28-moderate expression, KLK6 expression-non-metastasis) through Systems Biology/ Pharmacology tools.

The analysis included a variety of bioinformatics tools enabling the user to find important signaling pathways involved in breast cancer. Pharmacological targets have also been found in this way, some of which have not yet been studied for their criticality in breast cancer. Targets for parental cells are SRPK2 kinase, transcription factor GTF2I, LDL receptor, and oxidative phosphorylation. While for C5 cells the targets found are the transcription factor NRF1, autophagy and Unfolded Protein Response (UPR).

Keywords: KLK6, MDA-MB-231, Systems Biology/Pharmacology

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A bioinformatics approach to identify polymorphisms of genes related to iron metabolism that are also associated with susceptibility to infectious diseases

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Iron is an essential nutrient for both humans and pathogenic microorganisms that infect them. It is an indispensable metal that serves as a cofactor for many enzymes regulating vital cellular functions. Because iron is important for pathogens, human innate immunity has been evolved such as to deprive iron from pathogenic invaders, a procedure called nutritional immunity. In the present work we set out to identify all iron related genes that are statistically significantly associated with susceptibility to infectious disease. We identified 255 genes associated with iron metabolism from GO database. A PubMed literature search of all published meta-analyses on polymorphisms of these genes associated with infectious diseases resulted in 1228 articles of which only 53 fulfilled eligible criteria. Data recording included, among others, the metric Odds Ratio (OR), for all modes of inheritance, 95% CI, number of studies and participants. Statistically significant gene-disease association data were recorded from 118 meta-analyses comprising 1019 cases-controls studies with 181,897 cases and 304,752 controls. Twenty-one genes were found to be associated with one or more diseases caused by either bacteria, parasites, or viruses. Fourteen genes were found to be associated with bacteria caused diseases, seven with parasite diseases while only SLC11A1 was common. Finally, only two genes showed statistically significant association with viral diseases, while TNF was common with the bacteria infections-associated genes, summing up to 32 gene-infections pairs. Validation of the gene-infections association network (Cytoscape 3.0) created herein, with KEGG-Pathway enrichment analysis revealed that six gene-disease pairs (from 32) were common with KEGG, suggesting that the rest 26 could be considered as new updated entries in KEGG. Moreover, search for disease association of these 21 genes in KEGG database revealed 58 more associations (FDR 0.001) that we propose to be further investigated as iron metabolism related gene-disease associations.

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Genome-wide transcriptome analysis for the identification of chemoresistant-related genes in a DXR-resistant osteosarcoma cell model

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Chemoresistance is one of the most significant challenges in the treatment of cancer. It is defined as the tolerance of a cancerous tumor to the action of therapeutic agents. Unveiling the molecular mechanisms that regulate cells' responses to such agents, is of critical importance for targeting the development of chemoresistance. Osteosarcoma, or osteogenic sarcoma, is a primary malignant tumor of bones and is associated with the pathogenesis of mesenchymal cells. The incidence of the disease is 3 cases per million people per year, with these rates not having improved in the last 30 years. The etiology of the disease remains unspecified, but the most frequent genetic predisposition to osteosarcoma occurs in patients with retinoblastoma. In this study, genome-wide microarray data are analyzed, in order to identify chemoresistant-related biomarkers. For this, we used an *-in house* developed- osteosarcoma cell model, and normal human diploid osteoblasts (HDOs). This cell model was developed by the gradual exposure of three different osteosarcoma cell lines (U-2 OS, KH OS and Sa OS) to increasing concentrations of doxorubicin (DXR). This led to the development of two or three (depending on the cell line) different generations (referred to as R1, R2 and R3) of chemoresistant cell lines. The KH OS cells are characterized by mutated levels of the TP53 gene and decreased levels of endogenous CLU expression (a molecular chaperone related to chemoresistance). On the other hand, U-2 OS, express wild-type TP53 and RB1 genes and are characterized by high expression levels of CLU, while Sa OS cells are TP53 and RB1 null and express low CLU. Our bioinformatics analyses have revealed several differential gene expression patterns per cell line providing useful insights on likely chemoresistance biomarkers and thus possible novel therapeutic targets.

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¹H-NMR based metabolomic analysis of plasma, serum & urine in children with Growth Hormone Deficiency (GHD)

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Growth hormone deficiency (GHD) is a disorder caused by inadequate secretion of growth hormone from the anterior pituitary gland. GHD may be partial or total and may occur during infancy, due to genetic mutations or structural defects in the brain, or later in childhood, because of trauma, infection, radiation therapy or tumor within the brain. There is, also, a third category which has no known or diagnosable cause (idiopathic). Approximately, the disorder affects one in 4.000 to 10.000 children and characterized by abnormal slow growth, short stature, facial abnormalities, decreased bone mineral density and decreased energy levels. So far, diagnosis of GHD is considered a difficult and complicated procedure, which includes monitoring child's growth over a period as well as extensive biochemical and image exams. Metabolomics is an exciting scientific field, ideal for in-depth understanding of endocrinological disorders, especially in combination with Nuclear Magnetic Resonance (NMR) spectroscopy. NMR has been proven a powerful analytical tool for characterizing complex biological samples. Thus, through NMR based metabolomics, we aimed to investigate serum, plasma and urine metabolic profile of 26 children diagnosed with GHD and compare them with control group which consists of 33 healthy children. To visualize the GHD metabolic profile by NMR, analysis of metabolites in urine and blood samples was carried along with multivariate and univariate statistical analysis of the experimental data. The results of the study indicate differences at the levels of certain metabolites and give us the opportunity to examine in a very accurate manner the biological mechanisms which are involved.

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Prospecting novel and unexpected prokaryotic diversity in the sediment of Etoliko lagoon

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Etoliko lagoon is a unique landlocked lagoon in Western Greece with tectonic origin, hypoxic/anoxic conditions, high sulfate concentration and suffers from ecological problems. A previous single cell genomics study identified Etoliko Lagoon as one of eight unique environments worldwide with an extremely high representation of candidate phyla, exhibiting a unique and unexplored bacterial and archaeal diversity. The aim of this study was to further explore the prokaryotic communities in the sediment of Etoliko lagoon and identify potentially new taxa. Fieldwork was performed last year and a 70 cm core was retrieved from the deeper part of the lagoon (~27.5m). The core was divided in 14 layers, each 5 cm long. From each layer 5 subsamples of sediment were collected. Then the DNA of the subsamples was extracted using a commercial kit. The DNA quantity and quality was estimated and the hypervariable V3-V4 region of the 16S rRNA gene was amplified using universal primers for bacteria and archaea. The resulting amplicons were sequenced using a 2x300 bp pair end kit on MiSeq platform. Raw sequencing reads were demultiplexed, converted to FASTQ and then analyzed using custom bioinformatics pipelines. The prokaryotic communities were clustered according to their depth in the core. The taxonomy assignment revealed a large number of unassigned OTUs, even in phylum level and this may imply that the prokaryotes associated with Etoliko lagoon contained abundant potential novel taxa, which may be significant microbial resources. Those remained taxonomically unassigned bacteria and archaea may provide a better understanding in how microorganisms control global biogeochemical cycling of elements. Even more it will give an insight of the adapting mechanisms of the microorganisms in extreme environments and how those mechanisms can be exploited for biotechnological applications.

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In silico evaluation of Scutellarein as a potential inhibitor of the oligomerized A β -peptide in Alzheimer's Disease

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Alzheimer's Disease (AD) is a conformational, neurodegenerative disorder, characterized by cognitive impairment, and behavioral abnormalities. The neuropathology of AD lies in the presence of intracellular neurofibrillary tangles (NFTs) of the hyperphosphorylated tau protein and the existence of extracellular senile plaques, commonly known as amyloid plaques. The main component of amyloid plaques is the amyloid-beta (A β) peptide, derived from the amyloidogenic pathway of the proteolytic cleavage of the transmembrane Amyloid Precursor Protein (APP). Due to its tendency to self-aggregation and subsequent formation of protofibrils and oligomers, the soluble oligomerized A β -peptide exhibits substantial neurotoxicity and is considered as the crucial pathogenic species in AD. A novel therapeutic approach is the prevention of A β oligomerization by binding inhibitors to A β -peptide, to suspend its toxicity. It is believed that classes of compounds encountered in natural products, such as flavonoids, terpenoids and alkaloids, can play an imperative role in the battle against AD, due to their structural diversity, chemical and pharmacokinetic properties, as well as their ability to infiltrate the blood-brain barrier. Thus, the purpose of this study was to examine, by *in silico* methods, whether small chemical compounds could inhibit the aggregation of A β -peptide and subsequently, be suggested as future therapeutical agents.

Scutellarein, a flavonoid compound acquired from *Sideritis spp.* extracts, was subjected to Molecular Docking Studies and Molecular Dynamics Simulations with the NMR-derived structure of the oligomer of the A β -peptide₁₋₄₂. Upon analysis of the MD results, Scutellarein retained its topological affinity with the A β -peptide, forming a stable complex. In addition, scutellarein contributed to the modification of the peptide's conformation, which is inextricably linked to its pathogenic nature, by decreasing the number of amino acid residues of the A β -peptide participating in beta-strands. Therefore, scutellarein could potentially inhibit the aggregation of A β -peptide and be considered as a promising therapeutic agent against AD.

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Insights into the glyphosate-degrading enzymes C-P lyase and GOX using structural and molecular evolution analysis

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Bioremediation is the application of biological processes for the restoration of contaminated regions. It is a low-cost, sustainable and technologically simple clean-up method for ecosystems¹. The diversity and the presence of bacteria in different environments render them a constant source of study and research, aiming at their recruitment for the biodegradation of contaminants. Bioinformatics can contribute in this direction via the analysis of a vast amount of already available biological data². Our study focuses on the degradation of glyphosate, a widely used herbicide, by the bacterial enzymes C-P lyase and Glyphosate Oxidoreductase (GOX). We examined the conservation of C-P lyase and GOX across all major bacterial groups using the KEGG database for C-P lyase and BLASTp for GOX sequences. The conserved residues in C-P lyase were identified via MUSCLE multiple alignments and mapped onto the available 3-D structure of the enzyme using PyMOL. The BLASTp results for GOX were also assessed in terms of conserved residues. The 3-D structure of GOX was computed using Phyre2³, I-TASSER⁴ and AlphaFold2 Colab⁵, which were compared with PYMOL. Finally, molecular docking analysis of glyphosate on the two enzymes, can provide useful information about the interacting residues with the ligand. This approach can yield novel insights into the function of enzymes important for bioremediation.

Keywords: Biodegradation, Bioinformatics, glyphosate, glyphosate oxidoreductase, C-P lyase, multiple alignment, molecular docking

¹ Agrawal K, Bhatt A, Chaturvedi V, Verma P. Bioremediation: An Effective Technology toward a Sustainable Environment via the Remediation of Emerging Environmental Pollutants. In: *Emerging Technologies in Environmental Bioremediation*. Elsevier Inc.; 2020:165-196. doi:10.1016/b978-0-12-819860-5.00007-9

² Niranjana V, Reddy J, Suchithra V, Pooja R, Amshumala S. Role of informatics in bioremediation – A biological solution to environmental issues. *Int J Biol Res*. 2016;4(1):1-9.

³ Kelley LA, Mezulis S, Yates CM, Wass MN, Sternberg MJ. The Phyre2 web portal for protein modeling, prediction and analysis. *Nat Protoc*. 2015;10(6):845-858. doi:10.1038/nprot.2015-053

⁴ Roy A, Kucukural A, Zhang Y. I-TASSER: A unified platform for automated protein structure and function prediction. *Nat Protoc*. 2010;5(4):725-738. doi:10.1038/nprot.2010.5

⁵ Jumper J, Evans R, Pritzel A, et al. Highly accurate protein structure prediction with AlphaFold. *Nature*. Published online 2021. doi:10.1038/s41586-021-03819-2

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Gene Expression Meta-Analysis of Potential Shared and Unique Pathways between Autoimmune Diseases under Anti-TNF α Therapy

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The pivotal role of the tumor necrosis factor alpha (TNF α) in the pathogenesis of psoriasis (PsO), rheumatoid arthritis (RA) and inflammatory bowel diseases (IBD) has established the administration of anti-TNF α agents in the clinical routine¹. Despite the efficacy of anti-TNF α agents, several clinical trials have shown that 20-40% of patients do not respond to treatment². In this study, we performed a gene expression meta-analysis of patients with the above diseases under anti-TNF α therapy, in order to examine transcriptomic perturbations signatures between responders and nonresponders. Gene expression microarray datasets incorporated in our study were identified via literature search on the GEO database. We performed 4 meta-analyses, 3 disease-specific and a single combined, utilizing the random-effects model. We further identified the perturbed pathways through functional enrichment analysis and created an interacting network between the deregulated genes as derived from the combined meta-analysis. In total 9 datasets were used, with 4 referring to IBD, 3 to PsO and 2 to RA. Disease-specific meta-analyses unveiled about twice-fold number of deregulated genes in IBD in contrast to PsO and RA, where 11 out of those (3 up- and 8 down-regulated) were shared between each disease. Disease-specific pathways were associated with the interactions at the intestinal wall in IBD, keratinization in PsO and cell cycle in RA, while shared pathways between disease-specific meta-analyses referred to Neutrophil Degranulation and Signaling by Interleukins. Considering the combined meta-analysis, we uncovered 350 down-regulated and 86 up-regulated genes, which were significantly enriched for multiple inflammation-related pathways, including the Interleukin 10 pathway. Protein-Protein interaction networks between the deregulated genes of the combined meta-analysis elucidated the complex interactions taking place in the response to anti-TNF α therapy. Incorporation of regulatory elements, such as micro-RNAs, will enable a more holistic depiction of the mechanisms implicated in the response to anti-TNF α therapy.

¹ Wahren-Herlenius, M., & Dörner, T. (2013). Immunopathogenic mechanisms of systemic autoimmune disease. *The Lancet*, 382(9894), 819–831.

² Roda G, Jharap B, Neeraj N, Colombel JF. Loss of Response to Anti-TNFs: Definition, Epidemiology, and Management. *Clin Transl Gastroenterol*. 2016 Jan 7;7(1):e135. doi: 10.1038/ctg.2015.63. PMID: 26741065; PMCID: PMC4737871.r

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Meta-analysis of large-scale sequencing data (RNA-seq) to study the effect of abiotic environmental stress on rice (*Oryza sativa*)

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Rice is a staple food that provides nutrition to more than half of the world's population. Drought is a major abiotic stress that can have a significant impact on rice crop yield. As a result, identifying genes and gene products that are involved in a drought-resistance signaling system is critical. High-throughput technologies can aid in the detection of differentially expressed genes associated in drought-responsive rice biosynthesis. In the present study we investigated the impact of drought stress on two major rice subspecies, *ssp. japonica* and *ssp. indica*, by collecting all large-scale sequencing data available and conducting a meta-analysis. A systematic review was performed in GEO and PubMed databases to identify RNA-seq experimental data sets examining normal and drought-stressed rice samples corresponding of seedling/shoot tissue. Six out of 410 studies met the eligibility criteria and were further included in the meta-analysis. Using the newly created open-source tool for meta-analysis, MAGE, at a 0.5% significance level (FDR) we analyzed the six individual datasets and with meta-analysis we identified 1787 differentially expressed genes (DEGs). STRING database was used to study all possible protein-protein interactions, visualize results, and perform network analysis. Only 736 out of the 1787 proteins were found to be strongly connected (nodes) at an interaction score 0.7. Subsequently, functional enrichment analysis of the 736 gene products was performed with STRING and gProfiler. Ten proteins were found to be enriched in the biological process of cell redox homeostasis [GO:0045454]. The cellular component of the GO analysis showed two enriched categories [GO:0009507, GO:0009536] related to chloroplast/plastid regions with 67 participating proteins, along with respective membrane functions. Our findings constitute a cost-effective approach to identify drought-stress responsive genes that merit further experimental investigation for establishing stress tolerant genotypes.

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***Stevia rebaudiana* leaf extracts exert antioxidant activity that restores oxidative stress markers of experimentally diseased animals: a systematic review and meta-analysis**

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Stevia (*Stevia rebaudiana*) is a plant known to contain steviol glycosides (stevioside and rebaudioside) with significantly higher sweetening power than sugar. Emerging research and evidence suggest that stevia contains various other compounds with biological activities. Recent studies have shown several benefits of stevia consumption on human health such as antioxidant activity. Oxidative stress and Inflammation play pivotal roles in the pathogenesis of many diseases. Thus, in the present study we investigate, using meta-analysis, the way stevia affects oxidative stress markers [Catalase (CAT), reduced Glutathione (GSH), Superoxide dismutase (SOD), and Malondialdehyde (MDA)], in tissues of experimentally diseased rats that have been administered stevia leaf extracts. An inclusive literature search was performed to retrieve all possible studies investigating antioxidant activity of stevia on diseased rats. Datasets collection was performed according to PRISMA guidelines, and information was recorded regarding the type of oxidative stress marker (CAT, GSH, SOD, MDA), tissue, disease, and type of stevia extracts. Data were divided into three groups: 'control' (normal rats), 'case' (diseased rats) and 'stevia' (diseased rats that received stevia extracts). From the 138 articles initially retrieved from literature search, only 21 satisfied eligibility criteria containing 63 independent studies. Meta-analysis showed statistically significant differences between 'control' and 'case' and between 'case' and 'stevia' groups of rats, in all oxidative stress markers (CAT, GSH, SOD and MDA) groups. Importantly, CAT activity of 'stevia' group reached the activity of the control group (no statistically significant difference). Significant restoration of all other oxidative stress markers was seen for 'stevia' groups, reaching to about 80% the activity of the 'control' groups. Our results suggest that stevia leaf extracts can potentially act protectively against various diseases via its antioxidant properties. However, which of each of the multitude of stevia compounds, and to what extent, contribute to this effect awaits further investigation.

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Regulatory role of oxidative stress-induced lncRNA NORAD in cancer

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Long non-coding RNAs (lncRNAs), a class of transcripts with more than 200 nucleotides in length, they have various functions and engaged in multiple biological processes. Recently, are becoming increasingly recognized as key regulators of oxidative stress in several types of cancer. It has been discovered that oxidative stress causes DNA damage and causes apoptosis. Furthermore, multiple lncRNAs such as *MALAT1*, *BORG*, *NEAT1* and *NORAD* are also linked with treatment resistance and may possibly be promising biomarkers for predicting therapeutic outcomes. In this study, we present a transcriptomic analysis aiming to investigate the potential mechanisms involved in long noncoding RNA *NORAD* interactions. By sponging a downstream miRNA, *NORAD* could interact with the competitive endogenous RNA (ceRNA) network playing critical roles in a variety of biological processes, as well as, the development of neoplasms. Here, we utilized RNA-seq data from *NORAD* overexpression (shRNA) and knockdown (siRNA) experiments in A549 cells (lung adenocarcinoma) and HeLa (cervical carcinoma). Quantification of gene expression (TPM) and differential expression analysis were performed using salmon and DESeq2. The gene-set enrichment analysis for the differential expressed genes indicates that *NORAD* engaged in cell proliferation, development, and invasiveness. Furthermore, *NORAD* are associated and has binding relationships with multiple miRNAs (miR-22-3p, miR-618, miR-30c-5p, miR-552-3p, miR-202-5p, miR-877-3p, miR-495-3p, miR-345-3p). In addition, it has been shown that *NORAD* can enhance the autophagy and also, it can positively regulate *ATG5* and *ATG12*. It would be worthwhile to further investigate and explore the potential of stress-responsive lncRNA *NORAD* as biomarker in cancer treatment.

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A proteotranscriptomic-based analysis for target discovery and drug repurposing in Anaplastic Large Cell Lymphoma

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Objective: Design of a multifaceted pipeline for *in silico* therapeutic target and drug discovery in anaplastic large cell lymphoma (ALCL), based on integration of transcriptomic and proteomic data. Evaluation of the *in-silico* method via molecular biology, classical biochemistry, bioinformatics, and systems pharmacology.

Materials/Methods: The proposed method is based on the integration and optimization of publicly available proteomic and transcriptomic data, bioinformatic tools and algorithms, following a modular processing design adapted to the needs of lymphoid neoplasms. The current research consists of two parts: (a) *in-silico* modular analysis, including data selection, comparative analysis, pathway enrichment analysis, drug-repurposing and drug combination evaluation, and (b) *in vitro* analysis, including experimental evaluation of the action and effect of selected proposed drugs, employing a variety of available tools, such as immunoblotting assay, high content imaging and comparative transcriptomics and proteomics analysis.

Results: The application and evaluation of the proposed method in ALCL, identifies the transcriptional and proteomic characteristics and highlights the differences between healthy individuals and patients or ALCL subtypes. These differences were functionally characterized, classified, mapped to potential drugs (part a) remained to be confirmed experimentally (part b).

Conclusions: Multi-omics data analysis is an important aspect of cancer molecular biology studies that provides an integrated perspective of the pathogenesis of a disease, aiding the finding of putative pharmacological targets. Drug-repurposing is efficient, less expensive, less time-consuming, and riskless than traditional drug discovery. Computational and experimental analyses in combination, eliminate the disadvantages of each approach, facilitating the translation of results into clinical practice. The proposed pipeline could be applied to a variety of lymphoid neoplasms subtypes, to explore potential drug targets and to design more effective therapeutic protocols, considering the stage of the disease, or each individual patient's profile (precision medicine).

References

- ¹ Alan Talevi, 'Drug Repositioning: Current Approaches and Their Implications in the Precision Medicine Era', *Expert Review of Precision Medicine and Drug Development*, 3 (2018), 49-61.
- ² H. Xue, J. Li, H. Xie, and Y. Wang, 'Review of Drug Repositioning Approaches and Resources', *Int J Biol Sci*, 14 (2018), 1232-44.
- ³ B. McCabe, F. Liberante, and K. I. Mills, 'Repurposing Medicinal Compounds for Blood Cancer Treatment', *Ann Hematol*, 94 (2015), 1267-76.



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- ⁴ Elenitoba-Johnson KSJ (2017) Functional proteogenomics reveals biomarkers and therapeutic targets in lymphomas. *Proc Natl Acad Sci U S A* 114: 6581-6586
- ⁵ Zhang, X.-R., Chien, P.-N., Nam, S.-Y. & Heo, C.-Y. Anaplastic Large Cell Lymphoma: Molecular Pathogenesis and Treatment. *Cancers* vol. 14 (2022).

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aPEACH: automated pipeline for the analysis of ATAC- and ChIP-seq data

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With the advent of Next Generation Sequencing (NGS), experimental techniques such as the Assay for Transposase-Accessible Chromatin (ATAC-Seq)¹ and Chromatin Immunoprecipitation (ChIP-Seq)² have emerged as fundamental tools for studying the epigenome and transcription regulation on a genome-wide scale. The volume of generated data and underlying complexity regarding their analysis highlights the need for robust and easy-to-use computational analytic methods that can streamline the process and provide valuable biological insight. Our solution, aPEACH, is an automated pipeline that facilitates end-to-end analysis of ATAC- and ChIP-seq data, from downloading sample files to identifying signal-enriched regions (peaks) and annotating them. Our method is implemented in Python based on a modular approach that enables users to choose between well-established algorithms at each step of the analysis, such as TrimGalore for trimming, STAR³ for alignment and MACS⁴ for peak identification. aPEACH can either operate on locally stored data or it can automatically download samples from Gene Expression Omnibus (GEO) repository and organize the directory structure according to GEO accession metadata. The pipeline can process samples with single or multiple replicates. The latter case is typically suggested, since aPEACH can statistically measure the reproducibility of peaks with IDR⁵, as suggested by ENCODE⁶. A key and novel feature of our method is the accurate identification of the position within peaks with the highest signal coverage (summit), post-IDR application. IDR is highly inaccurate in finding peak summits, which can severely affect downstream analyses such as de novo motif discovery and characterizing differentially enriched loci. aPEACH also provides a variety of sample metrics such as quality control reports, fragment size distribution plots and all intermediate output files enabling the pipeline to be re-executed with different parameters or algorithms.

¹ Buenrostro, J. D., Wu, B., Chang, H. Y., & Greenleaf, W. J. (2015). ATAC-seq: A Method for Assaying Chromatin Accessibility Genome-Wide. *Current protocols in molecular biology*, 109, 21.29.1–21.29.9. <https://doi.org/10.1002/0471142727.mb2129s109>

² Robertson, G., Hirst, M., Bainbridge, M., Bilenky, M., Zhao, Y., Zeng, T., Euskirchen, G., Bernier, B., Varhol, R., Delaney, A., Thiessen, N., Griffith, O. L., He, A., Marra, M., Snyder, M., & Jones, S. (2007). Genome-wide profiles of STAT1 DNA association using chromatin immunoprecipitation and massively parallel sequencing. *Nature methods*, 4(8), 651–657. <https://doi.org/10.1038/nmeth1068>

³ Alexander Dobin, Carrie A. Davis, Felix Schlesinger, Jorg Drenkow, Chris Zaleski, Sonali Jha, Philippe Batut, Mark Chaisson, Thomas R. Gingeras, STAR: ultrafast universal RNA-seq aligner, *Bioinformatics*, Volume 29, Issue 1, January 2013, Pages 15–21, <https://doi.org/10.1093/bioinformatics/bts635>

⁴ Zhang, Y., Liu, T., Meyer, C.A. et al. Model-based Analysis of ChIP-Seq (MACS). *Genome Biol* 9, R137 (2008). <https://doi.org/10.1186/gb-2008-9-9-r137>

⁵ Qunhua Li, James B. Brown, Haiyan Huang, Peter J. Bickel. "Measuring reproducibility of high-throughput experiments." *Ann. Appl. Stat.* 5 (3) 1752 - 1779, September 2011. <https://doi.org/10.1214/11-AOAS466>

⁶ ENCODE Project Consortium (2012). An integrated encyclopedia of DNA elements in the human genome. *Nature*, 489(7414), 57–74. <https://doi.org/10.1038/naturepanagiota47>

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Comprehensive analysis of key long non-coding RNAs in chronic lymphocytic leukemia

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The long non-coding RNAs (lncRNAs) are functional biological molecules that are consisting of more than 200 nucleotides. Numerous studies support that lncRNAs play an important role in regulation of gene expression, the cellular response to stressful situations, and the emergence of a wide range of disorders. Because of their capacity to modulate the tumor microenvironment, they play a key role in tumor development, proliferation, invasion, and metastasis. Furthermore, the competitive endogenous RNA network (lncRNA-miRNA-mRNA) is associated to cancer initiation and progression in a variety of tumors. Chronic lymphocytic leukemia (CLL) is the most frequent type of leukemia for the adult population in the western countries. The CLL causes proliferation and accumulation of CD5+ B-lymphocytes in the peripheral blood, bone marrow, lymph nodes and spleen. CLL can be found in two forms, the aggressive (unmutated IGHV genes) and indolent (mutated IGHV genes). Latest studies have brought to light that epigenetic changes and ncRNAs alterations can change the outcome of the disease. The goal of this study was to investigate the key lncRNAs that are associated with chronic lymphocytic leukemia. For this analysis, we used RNA-seq data for 74 patients and 58 healthy individuals (Illumina sequencing, 100 base paired-end chemistry). Lightweight alignment and quantification of gene expression was performed with SALMON. Differential expression (DE) analysis was performed using DESeq2. In order to search for potential DE-lncRNAs – mRNAs interactions we used transcript sequences from starBase database. The key lncRNAs were annotated based on gene ontology gene-sets. Co-expression network analysis was conducted utilizing pairwise spearman's rank correlations. LncRNAs such as *MIAT*, *DLEU1*, *NEAT1* and *BM742401* were found to be associated with CLL. Oncogenic lncRNAs may one day be therapeutically targeted by a variety of methods, including post-transcriptional silencing, genome editing, suppression of RNA-protein interactions, even though there are still many obstacles to overcome.

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Investigating COVID-19 severity with NMR metabolomic analysis

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Almost three years after the first confirmed case in Wuhan, China, COVID-19 continues to negatively impact millions of lives worldwide. Despite the widespread implementation of vaccination protocols, disease burden remains high for patients and health systems alike. COVID-19 clinical manifestations range from asymptomatic to severe pneumonia and even acute respiratory distress syndrome (ARDS). Although a decrease in mortality rates has been recorded in the past year, being able to predict disease severity and clinical outcomes is still a matter of great interest. To this end, the metabolomics' approach could prove beneficial not only in investigating the biochemical background but also in establishing new predictive and diagnostic biomarkers to monitor disease progression and establish early prognosis. Utilizing NMR-based untargeted analysis, we aim to characterize the metabolic response of COVID-19 patients of different clinical severity. Serum samples obtained on the first day of hospitalization, from 60 patients requiring respiratory support for COVID-19 were analyzed. Severity was judged by the eventual need for non-invasive ventilatory (NIV) support and relevant clinical severity indexes, e.g., CCI, PSI, and SOFA scores. Multivariate statistical analysis was implemented to detect significant differences in metabolite levels between severe and non-severe groups. Our results confirm suspected alterations in metabolic processes as reflected by glucose, various amino acids, 3-Hydroxybutyrate, and lipid dysregulation in the group requiring NIV.

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2D/3D high-content screening analysis of DNA damage repair proteins using custom-made open-source tools

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Fluorescence microscopy analysis is commonly used in the DNA damage field to unveil the role of specific repair proteins in DNA repair pathways or characterize compounds in anticancer therapy. High-content screenings (HCS) allow the acquisition of multiple parameters at single cells, yet analyzing the data produced from HCS is challenging due to the large amount of data generated. Image processing, cell segmentation, identification of sub-nuclear structures and measurement of specific features should be conducted with robust algorithms, to be adaptable to the biological variability among the images. Analysis of such experiments is often performed by specialists due to data complexity. Therefore, developing tools under a user-friendly environment for automatic analysis is crucial. Here, we developed custom-made pipelines for automatic analysis of high-content 2D or 3D images. We show the application of this tool for automated analysis of several DNA repair factors in cells undergoing abnormal DNA licensing, a process that defines where along the genome and when during the cell cycle a given origin can fire. We analyzed recruitment of distinct repair proteins to damaged-DNA and identified sub-nuclear DNA damage foci colocalizing on specific genomic loci of cells undergoing aberrant licensing. Licensing deregulation has been linked to replication stress and tumorigenesis. Shedding light into the molecular mechanisms underlying these events could help unveiling novel targets for cancer therapy.

P155**Proteomics analysis reveals key regulators of Spinocerebellar Ataxia Type 1 molecular pathology****Sofia Notopoulou¹, Spyros Petrakis¹**¹*Institute of Applied Biosciences, CERTH, Thessaloniki, Greece*

Spinocerebellar ataxia type 1 (SCA1) is a hereditary neurodegenerative disorder caused by the presence of multiple (CAG) repeats in the *ATXN1* gene, encoding for the pathological polyQ (>35 Q) ataxin-1 protein. SCA1 has been linked with the selected loss of Purkinje neurons, resulting in severe movement dysfunction and eventually death within a decade after the onset. Despite the intense pathological phenotype, the underlying mechanism remains elusive and no treatment has been developed yet for this disease. Here, whole proteome analysis of human mesenchymal stem cells (hMSCs) inducibly overexpressing the *ATXN1* (Q82) gene revealed perturbation of metabolic pathways and elevated activity of the lysosome, whereas processes associated with RNA synthesis, surveillance and transport, along with ribosome assembly were significantly suppressed. Clustering of the most critical proteins into community hubs resulted in the identification of kinases and mediators which might be responsible for the dysregulation of the proteome. Finally, a computational analysis was employed for the prediction of drugs targeting these molecules; such drugs might reverse cellular pathology and rescue neurons from neurodegeneration.

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Disrupting the CARD11-BCL10-MALT1 signalosome complex to modulate immune response in glioblastoma multiforme

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Introduction

The CARD11-BCL10-MALT1 (CBM) signalosome complex triggers the adaptive immune response in lymphocytes and lymphoma cells via the integration of various receptor-induced signaling pathways that result in NF- κ B activation. Its aberrant activation has been associated with many NF- κ B signaling dependent neoplasms and immunodeficiencies. Herein, we mine the chemical space for MALT1 allosteric inhibitors as the MALT1-dependent cleavage of HOIL1 modulates canonical NF- κ B signaling (negative feedback termination), while the protease activity of MALT1 itself also controls NF- κ B signaling. Glioblastoma multiforme (GBM) serves as a paradigm.

Material and method

We employed a modular strategy; a. pharmacophore-based virtual screening, b. molecular docking-based virtual screening, both on a commercially available database of 7 million compounds and c. machine learning for drug repurposing on existing approved and experimental drugs. Upon data filtering, data analysis was performed with Datawarrior v.5.2.1 with clustering and similarity on the basis of the FragFP descriptor. Blood brain barrier permeability was predicted by LightBBB. Machine learning was employed on the drug repurposing information coming from DrugBank (~8.5k drugs). We put emphasis on the GBM immune landscape.

Results and discussion

Virtual screening and ML results were filtered based on scoring, BBB permeability and chemical diversity. Out of the final 13,178 candidate allosteric inhibitors, 55 chemical entities survived all strict criteria, while 48 of them were selected for efficacy and ADME-Tox screening in patient-derived glioblastoma cell lines.

Conclusion

GBM immunotherapy is challenged by tumour heterogeneity, limited immunogenicity and its immunosuppressive microenvironment. MALT1 allosteric inhibition serves a dual complementary role to treatment strategies.

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Integration of 1D and 3D data for in-silico drug repurposing in Diabetes Mellitus

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Diabetes mellitus affects more than 400 million people worldwide and this figure is expected to rise in the future. A well-established class of oral anti-hyperglycemic agents is Dipeptidyl-peptidase-4 (DPP-4) inhibitors. DPP-4 constitutes a therapeutic target in diabetes since it has a key role in glucose regulation. Yet, the existing DPP-4 inhibitors come with major drawbacks such as hypoglycemia, low potency and side effects due to lack of target specificity. Conventional drug discovery has failed to keep up with diabetes epidemic, while drug repurposing accelerates drug development. To this end we propose a novel *in silico* drug repurposing pipeline which incorporates heterogenous biochemical data and artificial intelligence towards the identification of novel DPP4-inhibitors.

The proposed methodology utilizes the 3D structures of DPP-4 and ~ 8.000 small molecules which were employed to perform molecular docking. The docking results generate a list of ranked repurposing candidates. Machine learning algorithms are used for the identification of false positive docking indications, prediction of drug-drug interactions and cytotoxicity prediction. Through Tanimoto similarity we evaluated the structural similarity revealed between the novel candidates and existing inhibitors. Next, those drugs that do not affect the same pathways as DPP4 were filtered out. Extensive filtering took place based on data from clinical trials as well as pharmacogenomics and pharmacovigilance information for the drugs in question. Molecular dynamics simulations, experimental bioactivity data from public repositories and gene expression data from Connectivity Map were used to identify the most potent candidates.

Our pipeline facilitates efficacy, safety, and selectivity ranking for candidate DPP-4 inhibitors.

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A machine learning model employing interaction fingerprint data for structure-based computational drug repurposing

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Drug repurposing is the process of uncovering new target proteins or indications of approved or abandoned drugs for use in a different disease. With the improvement of technology more and more computational tools are employed for drug repurposing. Such tool is Cloudfscreen platform, which is a 'one-stop-shop' platform for drug repurposing. *In silico* cheminformatics tools together with machine learning algorithms, are integrated in order to eliminate false positive pose results of molecular docking methodology. Approximately 11.000 protein structures were obtained from Protein Data Bank (PDB) and molecular docking simulations were performed with their co-crystallized ligands for 10 docking poses for each protein. Then, the root mean square deviation (RMSD) of the co-crystallized ligand with every docking pose of each protein was calculated, as well as an interaction fingerprint, that describes the interactions between the ligand and the protein. A variety of machine learning algorithms were trained and evaluated with the interaction fingerprint data and the model that showed the best performance was utilized to increase confidence levels in the results of the docking algorithm in new ligand-protein complexes. This machine learning application could pave the way for more confident predictions of molecular docking methodology in the field of drug discovery and drug repurposing.

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Synthesis and biological evaluation of chloramphenicol derivatives with modified dichloroacetyl tail

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Chloramphenicol (CAM) was discovered in 1947 and soon after became the first ribosome-acting and broad-spectrum antibiotic for clinical use, covering a wide range of Gram-positive and Gram-negative pathogens. Nowadays, despite the important therapeutic properties of chloramphenicol, its clinical use is limited due to the increasing resistance of the pathogens to CAM and its side effects. These facts have sparked an interest in the synthesis of new derivatives of chloramphenicol with improved pharmacological properties, i.e. molecules that will retain their antibacterial properties and at the same time reduce the toxicity and the resistance. In this light, we decided to combine alpha and beta amino acids with the CAM skeleton, by replacing its dichloroacetyl tail. For the synthesis of our library suitably designed for Structure Activity Relationship Studies (SARS), we conjugate the commercially available chloramphenicol base (CLB) with Trt or Boc N-protected amino acids or amino acids whose amino group is conjugated to dichloro- or difluoro- acetyl groups. The antimicrobial activity of all compounds was evaluated both in vivo and in vitro. According to the results, the bis-dichloroacetyl-ornithine-CLB derivative displayed the highest antimicrobial activity in both assays.¹

References

- ¹ Tsirogianni, A.; Kournoutou, G. G.; Bougas, A.; Poulou-Sidiropoulou, E.; Dinos, G.; Athanassopoulos, C. M., *New Chloramphenicol Derivatives with a Modified Dichloroacetyl Tail as Potential Antimicrobial Agents. Antibiotics (Basel)* 2021, 10 (4).

P160

Spectral characteristics of Color in *Lunaria annua* L. petals

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Lunaria annua L. is an emblematic flowering plant of Greek flora, family of Brassicaceae, with pink and purple to violet flowers having structurally complicated anthocyanin glycosides. The visible absorption spectrum of intact petals and of aqueous extracts shows a broad band with λ_{\max} centered at 560 nm with two shoulders, $\lambda_{\text{sh}} = 530$ and 603 nm. Acidified methanol spectra reveal a peak in the visible region around at 530 nm while a second peak in the UV-region at 320 nm is attributed to the acylation of sugar moiety with at least one aromatic organic acid. Concentrated acidified methanol extracts exhibit intense exciton interactions that disappear above 1/80 dilutions, with λ_{\max} shifting from 540 to 530 nm, and an absorption suppression of ca. 44X. Petal solubilization brings a huge (> 15x) increase in the $A_{\max \text{ uv}}/A_{\max \text{ vis}}$ ratio. Preparative HPLC of acidified methanolic extracts reveals two major peaks with low retention time, probably attributed to anthocyanin glycosides. These anthocyanins may present different colors, due to pH variations via dilution with freshly prepared buffers in the solution. In acidic media, the red flavylium cation is predominant with high absorbance and λ_{\max} around 520 nm. Pigment water extract is altered into a faint reddish color until pH 6, turns blue at neutral pH values where quinoidal anions are formed. The hydration reaction of flavylium cation which leads to colorless pseudocarbiniol acts competitively against ionization. Intramolecular copigmentation probably protects the molecule's chromophore(s) from discoloration. Addition of K^+ and Mg^{2+} to aqueous petal extracts leads to uniform spectrum absorption increases, while the addition of bi- or trivalent metals ions yields: (1) an hyperchromic effect and band broadening (Al^{+3}), a bathochromic spectral shift of ~ 30 nm (Fe^{+2}). Future work should determine the in-situ orientation of the flower chromophores.

Literature

- Griva S, Vaenas C, Mpadas P, Mpompotsialos N, Stefanidou G, Magoula M, Vrettos I, Kyrkas D, Mantzos N, Karagianni V, Beza EP, Papadopoulos GK. Characterization of anthocyanins with UV spectroscopy and TLC. The case of flower *Lunaria annua*. Poster, Proc. of 40th Conference of Hellenic Society of Biological Sciences (EEBE) Congress 2018, p. 90.
- Tatsuzawa F, Saito N, Shinoda K, Shigihara A, Honda T. 2006. Acylated cyanidin 3-sambubioside-5-glucosides in three garden plants of the Cruciferae. *Phytochemistry* 67:1287–1295.
- Trouillas, JC. Sancho-Garcia, V. De Freitas, J. Gierschner, M. Otyepka and O. Dangles (2016). Stabilizing and Modulating Color by Copigmentation: Insights from Theory and Experiment. *Chem. Rev.* 116, 4937–4982
- Brouillard R. (1983). The in vivo expression of anthocyanin colour in plants. *Phytochemistry* 22(6), 1311–1323.
- Harborne J.B. (1958). The chromatographic identification of anthocyanin pigments. *J. Chromatogr.*, Vol1:pp 473–478

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Flower color study of *Campanula versicolor* blue petal

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Campanula versicolor Andrews (1804: 396), is distributed in the southern Balkan Peninsula, in SE Italy and belongs to the family Campanulaceae. Petals from plants in the Region of Epirus are mostly blue/purple, sometimes white with a dark eye. We investigated the optical properties of petal flowers using spectrophotometry, TLC, and microscopy. We had previously reported asymmetrically localized pigment only in the upper epidermises, with intense petal coloration correlated with dense chromophore vacuolar concentrations. Furthermore, petal chromophores in-situ and in acidic methanol extracts exhibit exciton interactions with absorption suppression by a factor of ca. 10.

Of the various TLC systems of crude extracts tried, those with n-butanol:acetic acid:water (4:1:5) mixture as the mobile phase revealed a polar blue spot at the start line (delphinidine derivative) and a more apolar yellow spot, probably due to kaempferol. The visible spectrum of intact petal showed the characteristic triple maxima shape of many blue/purple flowers with peaks at 550, 585, and 632 nm. Such spectra very similar to those of crude extracts in water and 70 % ethanol, and to acidified methanol extracts of high chromophore density. The latter also showed nearby positive and negative peaks in near UV CD spectra. The appearance of a peak or shoulder in the 340-360 nm region of the absorption spectrum is consistent with the presence of yellow pigments, as flavanols, chalcones and xanthyllium salts. Complexation with added Mg^{+2} to aqueous extracts leads to slight absorbance increases without spectral distortions (peak ratios, peak and valley wavelengths), while additions of Al^{+3} produced mainly an hyperchromic effect, Fe^{+2} an intense bathochromic and hyperchromic effect with band broadening, and gross spectral distortions, strongly suggesting the presence of Mg^{2+} in the pigment vacuole. Work is in progress to isolate and identify the pigments, and their in-situ arrangement and metal chelation pattern.

Literature

Van der Kooij CJ, Elzenga JTM, Staal M, Stavenga DG. (2016). How to colour a flower: on the optical principles of flower coloration. *Proc. R. Soc. B* 283: 20160429. <http://dx.doi.org/10.1098/rspb.2016.0429>

Cristina Roquet, Llorenç Sáez, Juan José Aldasoro, Alfonso Susanna, María Luisa Alarcón and Núria Garcia-Jacas (2018). Natural delineation, molecular phylogeny and floral evolution in *Campanula* L. *Syst. Bot.* Vol. 33, No. 1, pp. 203-217.

Drakontaeidi A, Kafetzoglou K, Koromila I, Stefanidou M, Moustakas AK, Karagianni V, Kyrkas D, Patakioutas G, Papadopoulos GK. Molecular characterization of color pigments from the flowers of *Campanula versicolor* using spectrophotometric techniques. Poster presentation. 24th Biennial Meeting of the Hellenic Society of Horticulture. 20-24 October 2009, Veroia, Macedonia.

Brouillard R. (1983). The in vivo expression of anthocyanin colour in plants. *Phytochemistry* 22(6), 1311-1323.

Harborne J.B. (1958). The chromatographic identification of anthocyanin pigments. *J. Chromatogr.* Vol1:pp 473-478.

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The carob of Crete: Chemotaxonomic and bioactivity studies in *Ceratonia siliqua* samples of Cretan origin

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Carob tree (*Ceratonia siliqua*) is an evergreen tree of the Fabaceae family mainly met in the Mediterranean Basin. The nutritional value of carob is well known since ancient times, though somewhat neglected nowadays, in spite of the fact that it played an important role in the survival of the Cretans and the Spanish during the second world war and the civil war, respectively. Moreover, various parts of the carob tree may be useful in terms of their medicinal properties. Hence, there is a revived interest for carob tree cultivation, especially in warm Mediterranean regions like Crete. Since, literature data suggest that some activities of carob extracts are strongly influenced by gender and cultivar, our aim was to characterize samples from various Cretan *Ceratonia siliqua* cultivars in terms of their chemotaxonomic and bioactivity properties.

The initial chemical characterization of a cultivar originating from the area of Elounda (Eastern Crete) was based in the identification of secondary metabolites following cold extraction in either methanol/water or hexane and using NMR and ESI-MS. Clevenger distillation followed by GC/MS, analysis revealed the presence of twenty major volatile components including α -(Z,E)-farnesene, heptadecane, nonadecane, hexadecanoic acid, octadecanoic acid and E-phytol acetate. Compounds such as sucrose, glucose, fructose, pinitol and myo-inositol were identified and quantified in the extracts. Moreover, some of the extracts were found to possess selective cytostatic activity against cancer cell lines, as well as, significant antioxidant activity both in cell-free and in cell-based assay systems. Finally, activities related to skin ageing inhibition were also identified.

In conclusion, the chemical constituents and the biological properties identified in carob samples of Cretan origin are in line with literature reports concerning carob samples from other countries. Our aspiration regarding this ongoing effort is to provide chemotaxonomic and bioactivity data serving as a signature of each Cretan *Ceratonia siliqua* cultivar.

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The effects of organic excipients of insulin formulations on white blood cell viability, phagocytosis and cell migration

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Insulin injections are vital for millions of diabetic patients, improving their lives vastly. Microcrystalline insulin formulations are commonly used due to the stability and prolonged action they attribute; however, inflammation is a common phenomenon occurring at the infusion site of insulin in many patients.

Since the underlying mechanisms for acute skin irritation and inflammation are unknown, we aimed to examine the effects of the organic ligands, m-cresol, p-coumaric acid, 4-ethylresorcinol, 4-chlororesorcinol and 4-bromoresorcinol, on the viability of human white blood cells, on neutrophil phagocytosis, and on cell migration. The ligands were selected due to the desired crystal properties they attribute to insulin and the potential future use for the development of new insulin formulations. These molecules, which were used in concentrations ranging from 50 to 1250 μ M, exhibited mild differential effects on viability, phagocytosis and cell migration.

To conclude, further experiments assessing the effects of these ligands on phenomena closely associated with inflammation, such as the release of the pro-inflammatory cytokines, and the production of reactive oxygen species, are required.

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Identification of novel bioactive natural compounds using *in vivo* zebrafish phenotypic assays

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Identification of new Bioactive Natural Products (BNPs) that may serve as potential drug lead compounds is a constant challenge. Carrying out large-scale drug screens on mammals nowadays would be ethically and financially unjustifiable. Alternatives are offered by zebrafish embryos, which allow, in particular, *in vivo* monitoring of complex cell behavior and physiological parameters. Therefore zebrafish-based assays are gaining high popularity and wide usage in both academic and industrial drug discovery efforts as valuable whole animal platform for various stages of BNPs bioprospection.

One of the phenotype-based screen, aims to identify products related to pigmentation disorders. Abnormal pigmentation correlates with various aesthetic problems, as well as health diseases, including melanoma. We use the inhibition of melanogenesis during early embryo development to identify natural compounds that block melanogenesis. We have identified extracts from the Greek hawthorn *Crataegus pycnoloba* and from *Morus alba* as potent inhibitors of melanin synthesis and used activity-based fractionation to identify active subfractions and eventually single compounds that inhibit melanin synthesis. Finally, we identified the molecular mechanism via which their activity is mediated.

We are currently focusing on extracts and metabolites from several greek plants as well as macroalgae. Unlike terrestrial organisms that have been the subject of intensive research, marine benthic organisms are much less studied. Nevertheless, a number of secondary metabolites with valuable properties for the cosmetology sector have already been reported from macroalgae. Their active ingredients find applications worldwide not only in the food industry, but also in cosmetics. However, the rich biodiversity of the Eastern Mediterranean basin remains largely underexplored. We have identified extracts that promote wound-healing and we are screening to discover additional metabolites with anti-aging, anti-angiogenic, and melanogenesis-inhibitory activities.

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In Vitro evaluation of compounds of *Thymus thracicus* for inhibition of PTP1b, involved in Insulin receptor desensitization

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Anti-diabetic activity has been attributed to several *Thymus* species, among which *T. vulgaris* (1), *T. schimperi* (1) and *T. serrulatum* (2), mostly based on *in vivo* effects of *Thymus* extracts in animals. An effort to elucidate the mechanism of action of extract mixtures of *Thymus serpyllum* revealed increase in expression of glucose transporter GLUT 2, the Insulin receptor substrate 1 (IRS1) and the AMP-activated Protein Kinase (AMPK) all involved in insulin mechanism of action (3). However, the effect of specific ingredients has not been elucidated, while their effect on other factors such as the Protein Tyrosine Phosphatase (PTP1b) involved in insulin receptor desensitization has not been studied yet. Inhibition of PTP1b is known to improve insulin resistance, a key characteristic of Diabetes type II (DMII) and PTP1b has become a drug target for the treatment of the disease. Several plant ingredients have been found to act as PTP1b inhibitors (4,5). In the present study, seven compounds isolated from *Thymus thracicus* (6), were *in vitro* evaluated for PTP1b inhibitory action. The inhibitory action was measured by the p-nitrophenol colorimetric assay. Different substrate concentrations were used to elucidate the mode of inhibitory action. According to the results, four compounds showed characteristics of competitive inhibition. Four of the tested compounds (rosmarinic acid, calceolariside A, 9"-Methyl lithospermate and Dimethyl lithospermate) exhibited characteristics of competitive inhibition while two (2(3,4-dihydroxy) phenylethyl-glucopyranoside and Methyl caffeate) showed characteristics of uncompetitive inhibition. The 2(3,4-dihydroxy) phenylethyl-glucopyranoside exhibited the best inhibitory action ($IC_{50} = 21 \mu M$) while methyl rosmarinate presented no significant inhibition at any substrate concentration. The overall inhibitory action is comparable to that of other plant ingredients and could infer to a probable anti-diabetic action of the plant.

References

- Dessaiegn E. et al. Evaluation of In vitro Antidiabetic Potential of *Thymus schimperi* R. and *Thymus vulgaris* L. *Journal of Health, Medicine and Nursing*, 69, 9-16, 2019.
- Haile T, Cardoso SM et al. Chemical Composition, Antioxidant Potential, and Blood Glucose Lowering Effect of Aqueous Extract and Essential Oil of *Thymus serrulatus* Hochst. Ex Benth. *Front. Pharmacol.* 12, 621536, 2021.
- Azhar J, John P, Bhatti A. *Thymus serpyllum* Exhibits Anti-Diabetic Potential in Streptozotocin-Induced Diabetes Mellitus Type 2 Mice: A Combined Biochemical and In Vivo Study. *Nutrients*, 14, 3561, 2022.
- Bing TZ et al. Protein tyrosine phosphatase 1B inhibitors from natural sources. *Arch. Pharm. Res.* 41:130-161, 2018.
- Eleftheriou P. et al. Docking assisted prediction and biological evaluation of *Sideritis* L. Components with PTP1b inhibitory action and probable anti-diabetic properties. *Current Topics in Medicinal Chemistry*, 2019 DOI: 10.2174/1568026619666190219104430
- Papagrigoriou T. Pharmacognostic study of the *Thymus thracicus* Velen. *Plant. Master thesis, Thessaloniki, 2019.*

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Novel conjugates of mitoxantrone with leuprolide analogues that inhibit proliferation of breast cancer cells

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Leuprolide is a peptide-based agonist of the gonadotropin releasing hormone (GnRH) receptor. Its chronic administration leads to suppression of sex hormones secretion and thus, it is used for the treatment of estrogen-dependent breast cancer in premenopausal patients. Mitoxantrone is an anthracenedione antineoplastic cytotoxic chemotherapeutic agent that intercalates into DNA, resulting in cross-links and strand breaks and DNA and RNA synthesis inhibition. The aim of the present study was to study the effects of novel conjugates of mitoxantrone with leuprolide analogues on the proliferation of human breast cancer cell lines. Two human breast cancer cell lines, MCF-7 and MDA-231-MB, which are hormone-dependent and independent, respectively, were used. The effect of a range of concentrations of the conjugates on the viability of the cells was estimated by using the MTT assay in parallel with direct counting of the cells. Endocytosis of the conjugates was studied by fluorescence assays and their effect on cell apoptosis by Western blot analysis for PARP1 and caspase 3 cleavage. All conjugates decreased in a dose-dependent manner the proliferation of both MDA-231-MB and MCF-7 cells, at concentrations ranging from 10^{-7} M to 10^{-4} M. Leuprolide showed no effect alone and did not inhibit the conjugates' endocytosis or their effect on cell numbers. Data on apoptosis will be discussed. Our results suggest that some of the tested conjugates are effective against breast cancer cells in a pharmacologically relevant manner and more studies are in progress to elucidate their mechanism of action and to improve their efficacy.

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Association between different Biochemical and Oxidative stress parameters in dyslipidemia

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Dyslipidemia is associated with cardiovascular diseases. This study was conducted to estimate the relationship between different biochemical parameters with lipid profile. 100 volunteers, 50 males and 50 females aged 20-69 yrs participated in this study. They were divided in two groups, 40 participants with atheromatic index TC/HDL <3,9 (Group A) and 60 with atheromatic index TC/HDL >3,9 (Group B). Serum levels of different biochemical parameters such as HbA1c, Glucose, g Glutamyltransferase (γGT), Vitamin D (Vit D-25OH), total cholesterol (TC), triglycerides (Tg), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), alanine amino transferase (ALT), aspartate amino transferase (AST), lactate dehydrogenase (LDH), Alkaline Phosphatase (ALP), Potassium (K), Sodium (Na), Total Oxidative Status (TOS) and Total Antioxidant Status (TAS) were analyzed with colorimetric assays. The statistical analysis was performed by applying student t-test and Pearson's correlation coefficient p, at 0.0001 and 0.05 level of significance, respectively. In Table 1 it is depicted that Hb1c, Glu, Vit D, γGT, LDH and HDL-C were found to be significantly low, whereas lipid profile parameters except HDL-C, ALT, ALP and K had significantly higher values in participants with higher atheromatic index (Group B) comparing with those in Group A (p>0.0001). ALT, ALP, LDH, have shown significant positive correlation, whereas Vit D, AST, ALT, K have shown significant negative correlation with Reactive Oxygen Species (ROS) in all participants. ROS showed very weak association with atheromatic index and only LDL-C levels were positively associated with ROS levels especially in patients with lower atheromatic index (Group A). In conclusion, dyslipidemia seems to decrease serum Vit D, liver enzymes and K and only patients with high LDL levels present elevated ROS levels. Patients with dyslipidemia should frequently test Vit D, liver enzymes and K levels in order to prevent other diseases.



Biochemical Markers	Units	Group A Average Values	Group B Average Values
TC	mg/dL	151	240
HDL-C	mg/dL	48	52
LDL-C	mg/dL	78	159
TG	mg/dL	125	155
HbA1c	%	6,1	5,7
GLU	mg/dL	106	101
VITD-25OH	ng/mL	22	20
sAST	U/L	20	20
sALT	U/L	21	24
γ-GT	U/L	32	29
LDH	U/L	200	190
K	mmol/L	4,4	4,43
Na	mmol/L	141	141
ALP	U/L	64	73
TC/HDL		3,26	4,9
ROS	a.u	8513	6056
TOS	μmol/L	181,93	164,97
TAS	μmol/L	248,31	220,23
TOS/TAS		0,75	0,76

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Chemical compounds from *Humulus lupulus* (hop) exert anti-proliferative activity on cancer cells: a systematic review and a panoramic meta-analysis

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Humulus lupulus (Hop) is a dioecious plant belonging to the cannabinoid class. It is an indispensable component of beer since its polyphenols and α -acids give the characteristic bitter taste. Hop is a high added value agricultural product of economic interest in the agro-food sector, and recently has gained more interest due to biological properties, on human pathophysiology, such as cancer. In the present study a systematic review was performed to evaluate all assays measuring hop-induced growth inhibition of cancer cells. A total of 622 articles were retrieved from the literature search, and only 55 fulfilled all the inclusion criteria. As effect estimate the half maximal inhibitory concentration (IC_{50}) was recorded, together with standard deviation for each experiment. Sulforhodamine B (SRB), MTT, and Crystal Violet assays were the most employed. Subsequently, meta-regression based panoramic meta-analysis was performed, using STATA 13.1 software, to synthesize evidence of antiproliferative effect measured with each method, across studies, across compounds and cancer cell types for various time points. Meta-analysis showed that in every assay, the most effective compound to inhibit cell growth was Xanthohumol (IC_{50} values for Breast cancer: 10.4 μ M, Leukemia: 6.2 μ M, Colon cancer: 4.1 μ M), followed by α,β -dihydroxanthohumol (Breast: 9.1 μ M, Colon: 13 μ M) and Isoxanthohumol (Breast: 17.7 μ M, Colon: 28.2 μ M). The least antiproliferative effect was observed with Naringenin. In addition, every compound exerted higher cell-growth inhibitory effect on cancerous cells compared to normal cells. As expected, a reduction of IC_{50} was observed with time lapse from 24 to 72 hours. No specific effect for interaction between any compound and cell type was observed. In addition, SRB, MTT and CV methods gave comparable results, suggesting that they all could be used interchangeably for such screening. Further work is needed to systematically investigate the effect of other hop compounds, while hop extracts merit further investigation as potential bioactive natural products.

P169

Greek honey biological activities: comparative analysis of samples from various botanical and geographic origins

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Bee honey contains carbohydrates, proteins, vitamins, minerals, phenolic compounds and amino acids, contributing to its high nutritional and health value. The composition and the biological activities of honey vary significantly depending on its botanical and geographic origin, as well as, on the environmental conditions. Greece is one of the largest honey producers in Europe, while a wide variety of honey types is being produced in the country, due to its rich biodiversity. Aim of the present study was to characterize honey samples from various areas of Greece in terms of their antioxidant, atheroprotective and cytostatic properties.

This effort was part of the national Emblematic Action "The bee routes", and involved 49 honey samples supplied to us through the project's network. Viability of cells exposed to these samples was studied using the Neutral Red assay. The antioxidant activity of the samples was assessed using the ORAC (Oxygen Radical Absorbance Capacity) assay, while their atheroprotective capacity was studied based on their ability to deactivate oxidized low-density lipoprotein (LDL), to decrease reactive oxygen species (ROS) formation by endothelial cells EA.hy926 and to decrease TNF α secretion by macrophages THP1. Most of the samples studied were not affecting the viability of normal cells, while many of them were reducing the viability of human breast adenocarcinoma cells and to a lesser extent lung carcinoma ones. All samples were found to possess antioxidant activity, while two of the relatively efficient antioxidant samples were also found to deactivate oxidized LDL. Furthermore, two samples were found efficient in reducing ROS formation by endothelial cells and TNF α secretion by macrophages.

In conclusion, our data confirm the beneficial properties of Greek honey varieties for human health. Our effort along with those of the other Emblematic Action partners, will assist the in-depth characterization of each variety thus supporting the country's apiculture sector.

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Detection of protein aggregates in cellular models of SCA1 using Near-InfraRed fluorescence imaging

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Spinocerebellar ataxia 1 (SCA1) is a fatal neurodegenerative disorder, which belongs to the group of polyglutamine (polyQ) diseases. SCA1 is caused by trinucleotide (CAG) repeat expansions in the *ATXN1* gene and is associated with an expanded polyglutamine tract in the ataxin-1 protein. The expansion of CAG repeats in the *ATXN1* gene alters the conformation of the protein and leads to its misfolding and self-assembly into insoluble inclusions. Protein inclusions are mainly found in the Purkinje cells of the cerebellum; however, they are thought to be responsible for the widespread neuropathology of SCA1 disease. We have previously shown that insoluble inclusions induce oxidative and nucleolar stress. PolyQ inclusions contain fibrillar β -sheets potentially formed through polar zippers, which eventually assemble into amyloid fibrils. Therefore, the development of sensitive methods for the detection of protein aggregates may contribute to the early diagnosis of SCA1 and the development of therapeutic approaches against polyQ-induced oxidative stress.

The aim of this study is the detection of β -amyloids in polyQ ataxin-1 inclusions. To this end, we generated inducible Tet-On YFP-*ATXN1* (Q82) SH-SY5Y cells which accumulate polyQ inclusions and may be detected using β -amyloid-specific Near-InfraRed probes. We found that some of these probes detect amyloids compared to control SHSY5Y cells that do not contain protein inclusions. These results were further confirmed in assays using purified polyQ inclusions. This methodology may allow the monitoring of ataxin-1 aggregation which is responsible for the cellular oxidative stress.

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Assessment of the antibacterial and antioxidant properties of *Ailanthus Altissima* leave extracts

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Ailanthus altissima is an invasive species of the *Simaroubaceae* family, that has been introduced to the native flora of Greece. The fruits of *Ailanthus altissima* contain several bioactive compounds which may contribute to the development of new anticancer agents with potential applications in the cosmetics or pharmaceutical industry. Thus, in this study plant extracts were screened for their antioxidant, antimicrobial and cytotoxic properties. To prepare the extracts, leaves and barks were lyophilized, underwent tissue breakage, and the ethanolic and methanolic extracts were performed by using maceration, ultracentrifugation, and heat- and ultrasound-assisted extraction (HAE and UAE). *Ailanthus altissima* extracts were assessed for their antioxidant activity. The assays employed were (FRAP), ferric reducing antioxidant power, and scavenging effect on the 1,1-diphenyl-2-picrylhydrazyl free radical (DPPH). *Ailanthus altissima* extracts revealed significant effects in DPPH scavenging as well as hydroxyl radical scavenging activity and ferrous ions-chelating ability. The medicinal plants are known to contain number of phenolic compounds with strong antioxidant activity. Therefore, the total phenolic contents of the plant extracts were also determined spectrophotometrically according to Folin- Ciocalteau method.

Moreover, in this study, *in vitro* toxicological assessment of the *ailanthus altissima* extracts was performed in the human neuroblastoma cell line SK-N-SH. *Ailanthus altissima* extracts inhibited cancer cell proliferation and displayed cytotoxic activity in SK-N-SH neuroblastoma cells. The extracts of *Ailanthus altissima* exhibited also *in vitro* antimicrobial activity by agar disk diffusion method and MTT cytotoxic assay against *Klebsiella oxytoca* and *Debaryomyces spp.*

This work provides scientific supports for the high antioxidant and phytotoxic activities of this species and thus, it may find potential applications in the development of natural herbicides and antioxidants for agro-food and pharmaceutical industries.

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In vitro screening of extracts from the Greek flora as a basis for the development of innovative cosmeceuticals

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The unique biodiversity of the Greek flora – including a considerable percentage of endemic plant taxa – has triggered an increasing interest in the identification of bioactive compounds originating from these plants. We have focused especially on natural products with the ability to counteract one or more of the features of skin ageing, such as extracellular matrix degradation, increased reactive oxygen species, hyperpigmentation etc. The present work is part of a collaborative project that will eventually screen 104 plant extracts from different areas of Greece for biological activities necessary for the development of innovative cosmeceuticals.

The cytotoxic activities of the above extracts against human skin fibroblasts and keratinocytes have been assessed using two methodologies, the MTT and the Neutral Red assays, in order to identify the highest non-cytotoxic concentrations for further analyses. In parallel, the putative activities of the extracts against hyperpigmentation and against elastin degradation, were assessed in vitro using photometric assays and utilizing mushroom tyrosinase and porcine pancreas elastase, respectively.

The majority of the extracts were not cytotoxic at all (up to 100 µg/ml) for human skin fibroblasts and HaCaT keratinocytes (approximately 61% and 85% of the extracts for each cell type, respectively). For all other extracts the highest non-cytotoxic concentrations were determined ranging from 20 to 0.8 µg/ml. Regarding tyrosinase activity, it was found to be inhibited at over 50% by 15 out of the 104 extracts tested. Moreover, 20 out of 104 extracts were found to inhibit elastase activity by 50% or more. Some extracts were sharing both anti-tyrosinase and anti-elastase activities.

In conclusion, this initial biological profiling of extracts from the Greek flora indicates that they could support the development of novel and high-quality cosmetics.

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Antifungal effect and cellular localization of hyperbranched polymer nanoparticles in *Aspergillus nidulans*

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The increasing number of fungal infections is gradually becoming a major health issue, leading up to 1.5 million deaths annually. *Candida*, *Aspergillus*, and *Cryptococcus* species are responsible for the vast majority (90%) of deaths in immunocompromised and immunocompetent individuals [1,2]. Currently used antifungal agents exhibit toxicity, provide a narrow range of activity, and often encounter drug-resistance. Thus, nowadays the development of novel antifungal agents has become mandatory in order to overcome these limitations. Nanoparticles have occasionally been used as antimicrobials and antifungals [3,4], while dendritic polymers with functional terminal groups are able to target eukaryotic cells and, therefore, have the potential to be used as specific antifungals per se or as antifungal drug vectors. Here, we show that positively charged dendritic polymers are able to interact with the plasma membrane of the filamentous fungus *Aspergillus nidulans*, inhibit fungal cell growth and ultimately cause cell death. Hyperbranched polyethyleneimine (PEI) having molecular weight 25,000 Da and its guanidinylated (GPEI) and quaternized derivatives (QPEI) with a 50%-degree substitution of the PEI primary amino groups, prepared following previously described methodologies [3,5], efficiently inhibit fungal growth even at low μM concentrations. Fluorescence microscopy revealed that these nanoparticles labelled with fluorescent markers (FITC, Rhodamine) are located at the plasma membrane as well as the interior of both non-germinated quiescent conidia and germlings of the fungus. Treatment of cells with Propidium Iodide, a marker of dead cells or cells with compromised membrane integrity shows that PEI exhibits cytotoxicity at concentrations as low as 0.2 μM . The above-mentioned results indicate the antifungal potential of polyethyleneimine-derived nanoparticles, as well as the ability to "customize" them through their facile functionalized external groups to meet the desired properties.

¹ Brown, G. D., Denning, D. W., Gow, N. A. R., Levitz, S. M., Netea, M. G., & White, T. C. (2012). Hidden Killers: Human Fungal Infections. *Science Translational Medicine*, 4(165). <https://doi.org/10.1126/scitranslmed.3004404>

² Brown, G. D., Denning, D. W., & Levitz, S. M. (2012). Tackling Human Fungal Infections. *Science*, 336(6082), 647–647. <https://doi.org/10.1126/science.1222236>

³ Heliopoulos, N. S., Kythreoti, G., Lyra, K. M., Panagiotaki, K. N., Papavasiliou, A., Sakellis, E., Papageorgiou, S., Kouloumpis, A., Gournis, D., Katsaros, F. K., Stamatakis, K., & Sideratou, Z. (2020). Cytotoxicity Effects of Water-Soluble Multi-Walled Carbon Nanotubes Decorated with Quaternized Hyperbranched Poly(ethyleneimine) Derivatives on Autotrophic and Heterotrophic Gram-Negative Bacteria. *Pharmaceuticals (Basel, Switzerland)*, 13(10), 293. <https://doi.org/10.3390/ph13100293>

⁴ Lykogianni, M., Papadopoulou, E. A., Sapalidis, A., Tsiourvas, D., Sideratou, Z., & Aliferis, K. A. (2020). Metabolomics reveals differential mechanisms of toxicity of hyperbranched poly(ethyleneimine)-derived nanoparticles to the soil-borne fungus *Verticillium dahliae* Kleb. *Pesticide biochemistry and physiology*, 165, 104535. <https://doi.org/10.1016/j.pestbp.2020.02.001>

⁵ Lyra, K.-M., Kaminari, A., Panagiotaki, K. N., Spyrou, K., Papageorgiou, S., Sakellis, E., Katsaros, F.K., Sideratou, Z. (2021). Multi-Walled Carbon Nanotubes Decorated with Guanidinylated Dendritic Molecular Transporters: An Efficient Platform for the Selective Anticancer Activity of Doxorubicin. *Pharmaceutics*, 13(6), 858. <https://doi.org/10.3390/pharmaceutics13060858>

P174

Ataxin10 interacts with HIF-2 α and regulates its transcriptional activity

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Hypoxia-inducible factors (HIFs) are the master transcription factors that regulate cellular responses to hypoxia. The HIF family contains HIF-1 and HIF-2 that function as heterodimers, with an oxygen-regulated α subunit and a stably expressed β subunit, also known as ARNT. HIF-2 α is the less-studied isoform. Its expression is restricted to specific cell types and is involved in erythropoiesis, angiogenesis and metastasis. HIF-2 α is controlled so by oxygen-dependent as by oxygen-independent mechanisms, such as posttranslational modifications and interaction with other proteins. Its so far known interactions have not been sufficiently studied. Thus, we have researched for new HIF-2 α protein interactions. We have recently demonstrated that HIF-2 α interacts with Reptin52 and this interaction impairs HIF-2 transcriptional activity⁽¹⁾. In addition, we have distinguished a new protein that binds to HIF-2 α , which was identified by mass spectroscopy as Ataxin10. Ataxin10 is a protein that may function in cell survival, cytokinesis and differentiation.

In this study, to further investigate the involvement of Ataxin10 in the regulation of HIF-2 α , the expression of Ataxin10 was suppressed in HeLa cells by small interfering RNA (siRNA)-mediated silencing and we observed by RT-PCR reduced mRNA expression levels of the HIF-2-specific target genes such as PAI-1, CyclinD1, Catalase and Superoxide Dismutase 2, suggesting inhibition of HIF-2 transcriptional activity. In corroboration, Flag-Ataxin10 overexpression experiments led to an increase in HIF-2 transcriptional activity measured with Luciferase assay. Currently, we investigate the effect of HIF-2 α interaction with Ataxin10 in cells adaptation under hypoxia.

Our findings highlight Ataxin10 as a novel protein that regulates HIF2 activity, and their crosstalk remains to be tested.

Bibliography

¹ Gkotinakou et al., (2021) BBRC 143-150

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The interplay of $\Delta 133p53$ with $p53$ and its target genes in lung cancer

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$\Delta 133p53$ isoforms of the tumor suppressor gene $p53$, have been shown to be regulators of the cell cycle and apoptosis as they are activated in stress conditions. $\Delta 133p53$ isoforms along with the key partners of $p53$ and in particular the apoptotic factors MDM-2, PUMA and the cell cycle regulator $p21$, are considered to be responsible for the regulatory processes in cancer. Increased $\Delta 133p53$ levels have been found in various types of cancer including lung cancer.

The aim of the present study is to evaluate how changes in the expression of $\Delta 133p53$ isoforms, following overexpression or silencing experiments, affect the expression of both $p53$ and its target genes.

The human lung fibroblast cell line MRC-5 and the cancerous human lung alveolar epithelial cell line A549 were utilized in this study as in vitro models of normal and cancerous lung cell lines respectively. Overexpression and silencing experiments of $p53$ and $\Delta 133p53$ isoforms were performed. The effect of their overexpression as well as their silencing studied both at the transcriptional and translational levels, on $p53$ as well as on its target genes.

In the A549 cell line, the endogenous levels of $p53$, $\Delta 133p53$ transcripts were significantly higher compared to MRC5 cell line. The levels of $p21$ and $PUMA$ transcripts were substantially lower in A549 cell line. The overexpression of full length $p53$ transcript led to the upregulation of $p21$ and downregulation of $\Delta 133p53$ transcripts in A549 cell line. Similarly, silencing of full length $p53$ led to the downregulation of $p21$ transcripts in A549 cell line. The overexpression of $\Delta 133p53$ transcript promoted the downregulation of $p21$ whereas the levels of $PUMA$ transcript remained the same.

These results come in agreement with previous published data enhancing $\Delta 133p53$ mRNA level potential use as a biomarker in lung cancer.

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P176

The role of transcription factor TFAP2A in the hypoxic transcriptional control

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Cancer cells are exposed to hypoxic microenvironment due to their high proliferation rate and inadequate tumor vasculature. Hypoxia, via induction of HIFs, orchestrates reprogramming of gene expression that facilitates cellular adaptation. Sumoylation, covalent attachment of Small Ubiquitin related Modifier (SUMO) to proteins, has been lately implicated in the regulation of protein components of the hypoxic response. Using SUMO-immunoprecipitation in HeLa cells combined with quantitative proteomics we have identified several proteins, mainly transcription factors, with altered sumoylation status under hypoxia¹. One of the proteins with decreased sumoylation under hypoxia was TFAP2A, a transcription factor regulating a variety of cell processes including cell growth, differentiation and apoptosis. We have subsequently shown that TFAP2A physically interacts with HIF-1 α and its sumoylation status affects HIF-1 transcriptional activity¹. In order to characterize the interaction between TFAP2A and chromatin of hypoxia-inducible genes, we have performed ChIP-seq analysis using antibodies against TFAP2A or HIF-1 α . We could show that TFAP2A binds to promoters of a set of known hypoxic (mostly glycolytic) genes together with HIF-1 α . Moreover, silencing of TFAP2A inhibited their expression under hypoxia, suggesting that TFAP2A is a positive regulator of the hypoxic response. To further explore the involvement of TFAP2A in the transcriptional response to hypoxia, chimeric TFAP2A forms (wt, fully sumoylated, non-sumoylated) were overexpressed and their immunoprecipitates are being analyzed by mass spectroscopy. Our results so far suggest that TFAP2A may serve as a chromatin anchorage for HIF-1 α and/or facilitate the recruitment of transcriptional machinery components. As both TFAP2A and HIFs have critical roles in oncogenesis, revealing the functional significance of their interaction can lead to the development of novel strategies for targeting and killing cancer cells in hypoxic tumors.

¹ Chachami et.al. (2019) *Mol Cell Prot* 18, 1197-1209

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P177

Unraveling the role of N6-Methyladenosine RNA modification in response to DNA damage

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Like other major biomolecules, such as DNA and proteins, RNA molecules can be modified as well. The biochemical modifications of the RNA within a cell are known as the “epitranscriptome”. The most abundant RNA modification on RNA transcripts is the N⁶-methyladenosine (m⁶A), which has been shown to affect many biological processes, such as transcription, splicing and RNA metabolism, while dysregulation of its deposition has been linked to developmental abnormalities as well as various cancers. Recent findings suggest that m⁶A deposition on the RNA strand of R-loops regulates the resolution of those RNA:DNA hybrids, therefore, safeguarding genomic stability in human cells^[1]. However, the role of m⁶A modification on the R-loops and the mechanisms underlying this phenomenon, in response to DNA damage, have not been elucidated. In this study we present preliminary data of m⁶A-mediated response to damage reagents. These data give insights into the potent role of m⁶A modification upon DNA damage. Additionally, another aim of the study is the investigation of the role of m⁶A in R-loop formation. We report the progress of the development of MCF-7 breast cancer stable cell lines expressing inactive RNase H. In general, RNase H recognizes the RNA:DNA hybrids and catalyzes the cleavage of RNA, thus, ensuring genomic stability. However, inactive RNase H, binds the RNA:DNA hybrids without cleaving the RNA moiety. This is considered a useful tool for specific precipitation and visualization of R-loops. Cell lines expressing the inactive RNase H will help us examine the functional role of m⁶A deposition on the RNA:DNA hybrids in response to DNA damage.

¹ Abakir et al. Nat. Genet. 2020; 52:48–55

P178

Overexpression of the tRNA derivative 3'-tRF-Cys^{GCA} in HEK-293 cells leads to downregulation of epigenetic regulators

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tRNA derivatives constitute a class of small non-coding (ncRNAs) produced through specific enzymatic cleavage of tRNAs. These fragments have only recently emerged and, therefore, their function has not yet been fully elucidated. Some of them have been reported to interact with Argonaute proteins and, similarly to miRNAs, participate in the post-transcriptional regulation of protein-coding gene expression. Epigenetic regulators play a fundamental role in the control of gene expression by modifying the local state of chromatin. In this work, we aimed at examining the regulatory effect of 3'-tRF-Cys^{GCA}, a fragment deriving from the tRNAs bearing the cysteine (Cys) anticodon GCA. Based on our bioinformatic analysis of publicly available data from high-throughput RNA sequencing experiments after crosslinking immunoprecipitation (HITS-CLIP or CLIP-Seq), 3'-tRF-Cys^{GCA} is highly likely to target *HDAC2* and *CBX5*. Hence, the pCMV6 backbone was used to create a plasmid construct incorporating a tRNA^{Cys^{GCA}} gene, using restriction enzymes. The plasmid construct was transfected in HEK-293 cells using lipofectamine. The cells that received the construct were selected by treatment with G418. Next, DNA was isolated, and the incorporation of the plasmid construct in the genome was confirmed by PCR. Total RNA extraction was conducted, followed by polyadenylation and reverse transcription. The cDNAs were used as a template to conduct quantitative PCR (qPCR) assays, to assess the 3'-tRF-Cys^{GCA} and its putative target levels. Indeed, the overexpression of the parental tRNA has consequently led to the overproduction of its fragment; our qPCR experiments also showed that the mRNA levels of *HDAC2* and *CBX5* are lower in the three stably transfected clones, compared to the parental cell line, thus suggesting their tRF-mediated downregulation. Concluding, the attenuation of protein-coding gene expression through post-transcriptional regulation mediated by these RNA molecules could be exploited to silence epigenetic machinery players exerting an important role in the cells.

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Mechanistic insights into RNA-binding protein interactions that affect long non-coding RNA chromatin association

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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. lncRNAs can function in cis, while attached to their transcription site or near to it, or by interacting with other molecules such as RNA-binding proteins. In either case, lncRNAs are responsible for regulating the expression of target genes through various mechanisms. It has been observed that some lncRNAs transcribed from enhancer-like regions, are functioning in cis to regulate target gene expression¹, with the functionality coming from the chromatin-dissociated form¹. This suggests that release from chromatin is important in underlying the function of those lncRNAs, and thus regulation of target gene expression. Preliminary data predict RNA-binding proteins that interact with fast released lncRNAs. Some of those predicted candidates are the RNA processing factors *NONO* and *XRN2*, which are localized in chromatin and bind DNA, suggesting that they are involved actively in regulating this process. In this study, we present nanopore sequencing data concerning the effects on lncRNA chromatin/nucleoplasmic distribution following the silencing of *NONO* and *XRN2* in MCF-7 breast cancer cells. These data give insights into RNA-binding protein interactions that affect lncRNA's release or retaining. We also report the progress of the development of HepG2 liver cancer stable cell lines with functional depletion of these RNA-binding factors through the dTAG system. These cell lines are a potent tool in gaining mechanistic insights in the dynamics and fate of chromatin-associated lncRNAs and through downstream experiments we expect to help us pinpoint targets for locus-specific functional characterization.

¹ *Evgenia Ntini, Annita Louloui, Julia Liz, Jose M. Muino, Annalisa Marsico & Ulf Andersson Vang Ørom, 2018, Nat Commun, 9:1636*

P180

Assessment of immunostimulant activity of *Artemisia arborescens* and *Thymus vulgaris* extracts on gilthead sea bream (*Sparus aurata*)

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Fish infectious diseases are one of the main constraints of the aquaculture sector, representing a serious economic, social, and environmental challenge for the industry. The use of medicinal plants in aquaculture provides a sustainable way of protection using safe, eco-friendly compounds in a more cost effective way of treatment, compared to antibiotics and chemical compound currently used, due to their antimicrobial, immunostimulant, antioxidant, anti-stress, and growth-promoting properties^{1,2}.

Aim of the present study was the assessment of *Artemisia arborescens* and *Thymus vulgaris* feed supplementation effects on sea bream (*Sparus aurata*) immune responses. Fish were divided in 5 groups based on feed composition: a) control group - commercial diet, b) group 0.25% *T. vulgaris*, c) group 0.50% *T. vulgaris*, d) group 0.25% *A. arborescens* and e) group 0.50% *A. arborescens*. After two months of experimental fish *ad libitum* feeding, the effect of diets on fish weight and length were measured. Immunological parameters (i.e. nitric oxide, lysozyme, total protein) were determined on fish serum and/or mucus. Spleen samples were subjected to qRT-PCR to evaluate expression levels of genes related to antioxidants (SOD1, GPx1), cytokines (Il10, TGFb1, Il-1b, TNFa), antibacterial peptide (Hepcidin) and heat shock protein (GRP75). Fish weight and length in most diet groups showed no significant differences. Serum nitric oxide (NO) levels were increased in the 0.50% *T. vulgaris* group, while in 0.25% *A. arborescens* group the concentration of NO almost doubled compared to control group. The genes expression levels increased in both diets with high extract concentration. In the present study, the suitability of *A. arborescens* and *T. vulgaris* as efficient food supplements for immune status improvement was investigated. The results indicated that *A. arborescens* and *T. vulgaris* could be used as dietary supplements since they appear to have considerable potential as natural immunostimulants.

References

- ² Awad E., Awaad A., (2017). Role of medicinal plants on growth performance and immune status in fish. *Fish & Shellfish Immunology*, 67: 40-54
- ¹ Firmino J.P., Fernández-Alacid L., Vallejos-Vidal E., Salomón R., Sanahuja I., Tort L., Ibarz A., Reyes-López FE. and Gisbert E., (2021). Carvacrol, Thymol, and Garlic Essential Oil Promote Skin Innate Immunity in Gilthead Seabream (*Sparus aurata*) Through the Multifactorial Modulation of the Secretory Pathway and Enhancement of Mucus Protective Capacity. *Frontiers in Immunology*, 12: 633621.

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Mapping the role of RNase P protein subunits via CRISPR/Cas9 editing and NGS analysis

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Human RNase P, the essential ribonuclease for processing the 5' leader of precursor tRNAs consists of a sole catalytic RNA and ten protein subunits, some of which have been reported with moonlighting functions. The recent structure of the holoenzyme informs for detailed involvement of specific protein subunits in tRNA binding and catalysis, while the role of protein-protein interactions between specific subunits in the binding of other RNAs and their role in RNPs like RNase MRP, was also suggested. To clarify the essentiality or redundancy of each protein subunit we screened HeLa cells using the CRISPR/Cas9 tool for the role of POP1 (3 alleles) and RPP25 (2 alleles) subunits. Ablation of POP1 leads to lethality, thus confirming its central role as a core component of several important RNPs, beyond RNase P, RNase MRP, and telomerase (in yeast). In addition, ablation of RPP25 suggests that is dispensable for cell viability and although RPP25 knockout cells did not exhibit morphological alterations, the cell cycle is deregulated. Furthermore, *in vitro* biochemical assays showed that RNase P activity can still be detected in the edited cells, and moreover, NGS analysis showed that energy metabolism of cells and the RNA processing are altered through modulation of function of specific cellular compartments. Current and previous reports are supportive of the notion that specific individual protein subunits of RNase P although important for the overall architecture of the holoenzyme, can be dispensable for cell viability, raising questions on the minimum protein content of RNase P (and possibly other RNPs) under conditions like stress. Moreover, our observations highlight the possible alternative roles of RPPs beyond their participation in RNP complexes.

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Prognostic impact of 5'half-GlyGCC tRNA-derived fragment in multiple myeloma outcome and progression

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Despite the significant improvements in multiple myeloma (MM) therapy, current prognostic indicators cannot ensure optimal disease outcome, as high relapse rates and drug resistance remain significant challenges in MM's clinical course. Hence, there is urgent need for the elucidation of novel molecular markers aiding towards individualized prognosis and tailored therapeutics. tRNA-derived fragments (tRFs), rather than degradation debris, represent a novel group of small non-coding RNAs (ncRNAs) derived from tRNAs, that are implicated in a wide variety of physiological and pathological processes, including cancer initiation and progression. Herein, we have studied the clinical utility of 5'half-GlyGCC, derived from mature tRNA^{GlyGCC} in ameliorating MM risk stratification. Briefly, bone marrow aspirates were collected from 128 MM, 23 smoldering MM (sMM) and 16 monoclonal gammopathy of undetermined significance (MGUS) patients at diagnosis. Mononuclear cells were isolated using Ficoll-Paque, while CD138+ plasma cells were positively selected by magnetic sorting. Following RNA extraction and 3'-end polyadenylation, 5'half-GlyGCC levels were evaluated using *in house* developed RT-qPCR assays. Disease progression and patients' death were assessed as clinical endpoints for survival analysis. Our findings demonstrated the downregulated 5'half-GlyGCC levels in MM compared to its precursor stages MGUS and sMM (p=0.045). Intriguingly, within the MM cohort, higher levels of 5'half-GlyGCC were associated with inferior overall survival (HR=3.540, p=0.006) and higher risk for short-term progression (HR=1.901, p=0.045) of MM patients following treatment. Finally, multivariate Cox regression models unveiled the unfavorable independent prognostic value of 5'half-GlyGCC upregulation in patients' overall survival (HR= 3.100, p=0.028) when adjusting for MM patients' R-ISS stage, age, high-risk cytogenetics, response to 1st line therapy as well as LDH, B2M, and creatinine levels. Conclusively, we identified 5'half-GlyGCC upregulation in CD138+ plasma cells as an independent predictor of adverse disease outcome and contributed to better prediction of MM patients' early progression, supporting precision medicine decisions in patients management/monitoring.

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Rapid depletion of Scaffold Attachment Factor A (SAF-A) leads to distinct changes in nuclear actin

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Scaffold Attachment Factor A (SAF-A/hnRNP-U) is a highly abundant nuclear protein with a pivotal role in regulation of gene expression through modulation of the higher order structure of chromatin. It is considered one of the major nuclear scaffold/matrix binding proteins and possesses multiple binding domains such as for DNA, RNA and actin. Studies have shown that functionally, SAF-A, is implicated in the regulation of transcription, through interaction with RNA polymerase-II and actin [1]we set out to identify novel actin-binding proteins associated with RNA polymerase II (Pol II). Our group is aiming to better understand how the interaction of SAF-A with nuclear actin affects gene expression through modulating nuclear architecture. Hence, using CRISPR/CAS technology we have established a method by which we are able to quickly deplete SAF-A in human cells through an auxin-inducible degron (AID) tag [2]in order to study the immediate phenotypic consequences. Auxin-inducible degron (AID) and study the functional consequences on nuclear actin in real time.

Localization and mobility of different validated fluorescent actin visualizers were examined after SAF-A depletion. Surprisingly, disruption of SAF-A function or expression of a dominant-negative mutant of the protein, called C280, leads to a significant enrichment of wild type actin in the nucleus. Moreover, absence of the protein alters the distribution of both globular and filamentous nuclear actin individually. Monomeric actin seems to reorganize in the nucleus and polymerized actin is concentrating to small nucleoplasmic puncta after 24h, to a handful of much larger, brighter foci after 48h. Photobleaching experiments revealed that the protein's mobility was decreasing as the foci became larger. On the other hand, overexpression of SAF-A leads to accumulation of endogenous globular actin in splicing speckles, nuclear domains enriched in pre-mRNA splicing factors and involved in transcription machinery.

¹ A. Kukalev, Y. Nord, C. Palmberg, T. Bergman, and P. Percipalle, "Actin and hnRNP U cooperate for productive transcription by RNA polymerase II," *Nat. Struct. Mol. Biol.*, vol. 12, no. 3, pp. 238–244, 2005

² T. Natsume, T. Kiyomitsu, Y. Saga, and M. T. Kanemaki, "Rapid Protein Depletion in Human Cells by Auxin-Inducible Degron Tagging with Short Homology Donors," *Cell Rep.*, vol. 15, no. 1, pp. 210–218, 2016

P184

The role of HSP90 in sam organization and functiony during reproductive phase transition in *Arabidopsis Thaliana*

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The timing of flowering is regulated by environmental and endogenous signals that converge at the Shoot Apical Meristem (SAM). During phase transition, SAM takes on an inflorescence meristem (IM) identity that balances two antagonistic processes: to remain undifferentiated and at the same time to continuously produce new cells, which are incorporated into new flower primordia. The fate of meristematic cells confers the developmental plasticity exhibited by plants throughout their life cycle. However, little is known about the molecular processes controlling meristem cells' responses to environmental stimuli. Heat Shock Protein 90 (HSP90) is an evolutionary conserved molecular chaperone that modulates many cellular processes under physiological and stress conditions. It's role in regulating developmental plasticity and environmental responses in plants is well established. Previously, we showed that cytoplasmic HSP90s are expressed in the SAM under normal and stress conditions [1] and that they are essential for the transition from vegetative to reproductive state and for flower meristem formation [2]. To investigate further the involvement of HSP90 in this process, we examined the SAM organization and size of *hsp90^{RNAi}* knock down plants under different photoperiodic regimes. Our findings show that HSP90 depletion results in abnormal morphological features and size of the SAM. In order to characterize the developmental stage of the plants, we performed expression analysis of genes that promote flowering transition and confer flower primordia identity. The expression pattern of the genes involved in both processes were altered, predominantly those involved in the flowering pathways of photoperiod and age. A modified spatiotemporal expression was also observed for SPL9 which is a key regulator of the transition from vegetative to reproductive development. Furthermore, we investigated the putative interaction of HSP90 with REF6 and SYD, two protein factors that control gene expression and SAM development through epigenetic regulation of their targets. Our data shed light into the fine-tuning mechanisms that control the delicate function of the SAM, which is vital for proper development and seed production.

¹ Prasinos et al., (2005). Tight regulation of expression of two *Arabidopsis* cytosolic Hsp90 genes during embryo development, *Journal of Experimental Botany*, Volume 56(412), 633–644. <https://doi.org/10.1093/jxb/eri035>

² Margaritopoulou et al., (2016). HSP90 canonical content organizes a molecular scaffold mechanism to progress flowering. *Plant J*, 87: 174-187. <https://doi.org/10.1111/tpj.13191>

P185

Effect of simvastatin on the expression of cellular receptors in breast cancer cells and their contribution to patients' survival

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Breast cancer currently has the highest occurrence and cancer-related death rates among women. Despite the fact that multiple breast cancer treatment plans are being followed, they are at times ineffective, followed by unpleasant side effects for the patients. Thus, it is of great importance that supplemental drugs must be developed to prevent overtreatment with chemotherapy so as to improve the quality of life of patients during treatment as well as the effectiveness of the main treatment itself. It has been observed that the inhibition of the mevalonate pathway by statins induces pleiotropic effects on the cells besides the reduction of LDL cholesterol in the serum. That includes the regulation of signaling molecules that affect breast cancer cell proliferation and survival.

In the present study the effect of simvastatin on the expression of selected cellular receptors of breast cancer cells is studied. It was found that: a. PR followed a dose dependent increase in MCF-7 cells, whereas remained stable and in negligible amounts in MDA-MB-231 cells, as it is expected, b. AR followed an almost dose-dependent increase in MCF-7 cells, but an almost dose-dependent decrease in MDA-MB-231 cells, c. GPER showed an increase in intermediate concentrations of simvastatin in both cells, followed by an increase at the high concentration examined, and in all cases its expression was higher than that of controls. The results suggested a different effect of simvastatin on receptor expression from breast cancer cells.

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Effect of lumican on breast cancer patients' survival - Effect of simvastatin on lumican expression in breast cancer cells

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Breast cancer is the most common cancer among women worldwide with an incidence of 25% and it is the fifth cause of death among women worldwide. The high incidence of the disease suggests that new methods are required to improve its prognosis, diagnosis and treatment, especially for the most aggressive type, the triple negative (TNBC).

An important subfamily of the proteoglycans is the small leucine rich proteoglycans (SLRPs), which are present in the extracellular matrix (ECM) and provide structural support and organization and thus potentially regulate cancer cell proliferation, angiogenesis and metastasis. Their relatively small molecular mass and the presence of common structural motifs, such as leucine-rich repeats (LRRs), are their main characteristics.

Statins belong to a widely used anti-lipidemic drugs for preventing cardiac disease and their efficacy is well established. However, studies suggested that statins may influence cancer growth, through their pleiotropic activity.

The aim of the present study was the examination of the effect of lumican in breast cancer patients' survival through data mining and the effect of simvastatin on lumican expression using two commonly used breast cancer cell lines, MCF-7 and MDA-MB-231.

Our results showed that lumican expression was slightly increased in cancerous samples, compared to normal, although in patients with TNBC there was a clear decrease in expression. In addition, the high expression of lumican had a beneficial effect on OS of Luminal B cancer patients and of DFMS of TNBC patients. Simvastatin affected expression of lumican in cells differently. It suppressed expression in MCF-7 cells up to 70% in a dose-dependent manner, whereas it increased expression in MDA-MB-231 cells in all concentrations examined. These results suggested that simvastatin might be used only for treatment of TNBC patients.

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The chromatin insulation activity of the retinoblastoma tumor suppressor (RB)

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The retinoblastoma tumor suppressor (RB) is a well characterized cell cycle regulator; RB associates with E2F and inhibits the expression of genes essential for cell cycle progression. Lately, additional cellular functions have been associated with cell cycle-independent RB activity, but their mechanisms are poorly understood. Our studies revealed that RB association with human chromatin is not restricted to E2F promoters. Interestingly, we defined distinct pools of chromatin-bound RB in enhancer regions occupied by AP-1, or CTCF-bound loci that lack both enhancers and promoters' marks and are enriched in components of the cohesin complex. Thus, RB can be engaged in different mechanisms of chromatin regulation to target distinct sets of genes. This new approach to exploring RB-mediated chromatin regulation could provide mechanistic insights into RB functions beyond the orchestration of the E2F program.

Interestingly, 80% of the regions co-occupied by RB and CTCF are Topological Associated Domains (TADs). Therefore, we hypothesized that RB is involved in regulating the chromatin interactions in RB/CTCF-bound chromatin loci. Specifically, we asked whether RB-mediated regulation of chromatin boundaries is cell cycle-dependent and if the RB-dependent chromatin loops associate with the regulation of gene expression. For this, we performed Micro-C, RNA-seq, and proteomic analysis in cell cycle synchronized RB1 wild-type and RB1 CRISPR knock-out RPE1 cells. Our data demonstrate that depletion of RB does not significantly change the protein levels of CTCF and the cohesin complex components. Indeed, CTCF and cohesin association with chromatin are cell cycle and RB-independent. However, we identified that the cohesin complex broadly overlaps with RB-bound promoters and enhancers in accessible chromatin regions. When cells accumulate in G1, there is an RB-dependent inhibition in chromatin interactions. Our future studies will focus on how the RB-mediated chromatin loops associate with the activity of the RB-bound promoters and enhancers and the RB-dependent regulation of transcription.

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Pre-operative serum miR-205 levels in bladder cancer outcome

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Bladder cancer (BlCa) constitutes a major cause of cancer-related morbidity and the most expensive per-patient-to-treat malignancy, due to the lifelong and highly invasive surveillance of the patients. Accurate disease prognosis is crucial in establishing personalized therapeutic decisions; yet optimum tools for precise risk stratification remain elusive due to the high molecular and clinical heterogeneity of bladder tumors. Nowadays, liquid biopsy approaches, such as the evaluation microRNAs (miRNAs) in patients' circulation, hold great promise as new minimally invasive strategies, aiming to ameliorate modern real-time cancer management. Herein, in our study, we have highlighted the clinical utility of pre-operative serum miR-205 levels in improving BlCa patients' prognostication. The study's screening cohort consisted of 108 patients diagnosed with primary BlCa, while serum samples were obtained prior to transurethral resection of the bladder tumor (TURBT) for non-muscle-invasive bladder cancer patients (NMIBC; TaT1) or radical cystectomy (RC) for muscle-invasive bladder cancer patients (MIBC; T2-T4). In brief, following circulating miRNAs extraction and 3'-end polyadenylation, miR-205 levels were quantified by *in-house* qPCR assays. Patients' survival outcome was assessed using progression for NMIBC and patients' metastasis and/or death for MIBC as clinical endpoint events. Bootstrap analysis was performed for internal validation of Cox regression analysis. Interestingly, elevated serum miR-205 was correlated with muscle-invasive disease ($p < 0.001$) as well as with tumors of higher stage ($p < 0.001$) and grade ($p < 0.001$). Moreover, the survival analysis of our screening cohort unveiled the significant association of increased serum miR-205 levels with early progression (HR=4.650, $p=0.026$) of NMIBC patients and inferior disease-free survival outcome (HR= 2.094, $p=0.037$) of MIBC patients following surgery. In conclusion, our study identified increased pre-operative serum levels of miR-205 as a powerful non-invasive predictor of BlCa patients' post-treatment progression, augmenting prognostic accuracy and supporting precision medicine decisions in BlCa management.

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Dissecting the role of a putative DEAH-box RNA helicase 35 (DHX35)

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Helicases are ubiquitous enzymes present in all kingdoms of life and are essential in all aspects of nucleic acid metabolism. Helicases are mainly involved in unwinding specifically DNA, RNA or DNA-RNA hybrids and remodeling ribonucleoprotein complexes. Based on our previous work, we identified a putative ATP-dependent RNA helicase implicated in ribosomal RNA synthesis and processing, termed DEAH-box polypeptide 35 (DHX35). Interestingly, we detected a diverse expression profile of DHX35 not only in melanoma and lung cancer cell lines but also in samples derived from patients. This observation prompted us to further characterize DHX35 to unravel its biological function and mechanistic role in the malignancy onset and progress. Therefore, we first cloned and overexpressed DHX35 in an E. coli heterologous system. Recombinant DHX35 was then purified by size exclusion chromatography and was tested for substrates binding. Of note, DHX35 appears able to bind specific RNA molecules, and in particular snoRNAs (e.g. SNORD78 and SNORA73B) and precursor tRNAs as we showed by conducting Electrophoretic mobility shift assay. However, DHX35 could not bind any DNA substrate. Finally, we further achieved to knock out DHX35 in HeLa cell line by using CRISPR/Cas9 genome editing tool in an effort to thoroughly divulge the DHX35 biological role.

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P1 tRNA^{Gly} ablation from *Staphylococcus aureus* deregulates iron homeostasis, tRNA pool and susceptibility to antibiotics

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Besides the established role of tRNA in translation, it has been currently proven to possess additional non-canonical functions crucial for bacterial homeostasis and pathogenicity. Our previous work revealed the tRNA-mediated synchronization of translation and cell wall synthesis in the human pathogen *Staphylococcus aureus*. Specifically, five distinct tRNA^{Gly} isoacceptors exist in this pathogen, two of which are specialized to participate to protein translation and are called proteinogenic (P1, P2), while three are termed non-proteinogenic (NP1, NP2, New) and contribute to cell wall stabilization. Moreover, all tRNAs^{Gly} regulate the transcription of the glycyl-tRNA synthetase (GlyRS), an essential enzyme responsible for charging tRNAs^{Gly}, through binding to a structurally unique *glyS* T-box riboswitch. Interestingly, we showed that mainstream antibiotics bind to this special tRNA^{Gly}:*glyS* T-box complex and affect diversely the expression of GlyRS, highlighting a novel appealing molecular strategy against staphylococcal infections. Therefore, in order to unravel in-depth the role of each tRNA^{Gly} we knocked out the proteinogenic P1 tRNA^{Gly} by utilizing the CRISPR/Cas9 genome editing system. Although the edited strain grows similarly to the wild type, it appears more susceptible to antibiotics which prominent target is the cell wall. Interestingly, transcriptome analysis of the edited strain revealed differential expression of genes implicated to the iron homeostasis, which is a key process for bacterial infectivity and pathogenicity. Although, the non-proteinogenic tRNAs^{Gly} are upregulated, genes essential for the cell wall formation and the *glyS* T-box are downregulated. The purine metabolism and the redox status are also affected. Remarkably, variations of other tRNA species expression and an increase in the non-proteinogenic tRNA^{Gly} halves are observed. Our results present the basis of a tRNA-mediated interconnection of distinct cellular processes that could be further exploited to fight species-specific infections.

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P191**Effect of pharmaceutical and dietary products in EGFR and IGFR expression in breast cancer cell lines****M. Anastasiou, Th. Rapti, M. Kanellakis, D.H. Vynios***Biochemistry, Biochemical Analysis & Matrix Pathobiochemistry Research Group, Department of Chemistry, University of Patras, 26504 Patras, Greece*

The function of each cell in a multi-cell organism is the combined effect of endogenous programming and interactions with both neighboring cells and the environment. Growth factor receptors EGFR and IGFR belong to one class of proteins that contribute to this process. Cancer is a disease characterized by uncontrolled proliferation of cells. Specifically, the activation of the signaling pathways of these receptors has been proved crucial in the development of the disease.

The benefits of statin treatment for preventing cardiac disease are well established. However, studies suggested that statins may influence cancer growth. Their action leads to disruption of the cell membrane, affecting molecules such as transmembrane receptors, EGFR and IGFR.

Stevia is used as a noncaloric sweetener in several countries. Beyond their value as sweetener, the steviol glycosides contained may also offer therapeutic benefits as they have anticancerous properties. In particular, steviol, the aglycone portion of the glucosides, appears to affect the expression of these receptors.

The present study aims to evaluate the biological activity of statin and steviol glycosides in breast cancer cells, and in particular on the expression of growth factor receptors, EGFR and IGFR, where it was found an induction to changes in gene expression of these receptors.

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The effect of triple mutants of transcriptional adaptors in the expression of genes related to the transport and biosynthesis of auxin in *Arabidopsis thaliana*

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Histone acetylation is one of the most important post-transcriptional modifications, regulating the unravelling of the DNA from the histone octamer and allowing transcriptional initiation. SAGA is a multiprotein complex with a histone acetyltransferase module (HATm). In yeast, the HAT module consists of acetyltransferase GCN5 (GENERAL CONTROL NON-REPPRESSED PROTEIN 5), ADA2, ADA3 and SGF29. In *Arabidopsis thaliana*, the HATm contains two ortholog proteins for each ADA2, ADA3 and SGF29. GCN5 and ADA2b appear to be required for developmental processes and various responses to environmental cues. Single *gcn5-6* and *ada2b-1* mutant plants exhibit pleiotropic phenotypes, including dwarfism, reduced root length and abnormalities in flower development. Triple *ada3a-2;ada3b-2;ada2b-1* and *ada3a-2;ada3b-2;gcn5-6* mutant plants exhibit aggravated phenotypes in comparison to the single mutants and double *ada3a-2;ada3b-2* mutants. The more severe phenotype of triple mutants suggests an interaction between these components of the HATm. Therefore, we studied the expression of genes involved in auxin biosynthesis and transport in single, double and triple mutants of three members of the HATm. The gene expression of auxin efflux carriers *PIN3* and *PIN4* was similarly reduced in single, double and triple mutants, suggesting that GCN5, ADA2 and ADA3a/b function together as positive regulators. The expression of the first auxin biosynthesis gene *TAA1* was also reduced in all mutants tested compared to wild-type plants. In contrast, the transcript levels of *YUC5* and *YUC8* were increased in the mutants tested, indicating that auxin biosynthesis is also affected by members of the HATm. Our results suggest that GCN5, ADA2b and ADA3a/b regulate auxin responses by affecting the expression of genes involved in biosynthesis and transport.

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Assigning efficacy and specificity of HuR-disruptors in inflammation

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The innate immune response involves a variety of inflammatory reactions that can result in inflammatory disease and cancer. The effective activation and resolution of immune responses rely on regulating mRNAs encoding inflammatory effectors. The RNA-binding protein, HuR/ELAVL1 controls the post-transcriptional fate of inflammatory and oncogenic mRNAs, facilitating inflammatory and degenerative pathologies. As such, it has prompted the search for inhibitors to interfere with its biological activity in inflammation and cancer. In this study, we set up a standardized cellular platform to screen five presumed disruptors of HuR:RNA interactions (DHTS-1, CMLD-2, quercetin, resveratrol and AICAR) for their efficacy in altering the responses of activated or polarized macrophages. Specificity was assessed by integrating primary macrophages from transgenic mice harboring debilitating mutations in the gene of HuR; and in genes of known functional interactors/antagonists like AUF1/hnRNP D, TTP, and TIA-1. According to our results, only one compound (DHTS-1) could interfere with inflammatory responses in a stoichiometric and HuR-specific manner, validating the functional interaction between these two molecules. Conversely, other compounds showed partial HuR dependency (CMLD-2) or acted in a HuR-independent manner. Overall, our findings highlight the need for specialized cellular platforms to assess the efficacy and specificity of post-transcriptional regulators; and the outcome of their proper targeting in inflammation control.

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Expression control of chondroitin synthases and dermatan sulfate epimerase in breast cancer cells in the presence of simvastatin

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Proteoglycans are among the most important functional and structural biomolecules in cells and tissues. They consist of a central protein substituted with one or more glycosaminoglycans. Their presence at the extracellular space makes them of great importance not only in tissue structure, but in cell functional properties. The involvement of many enzymes with different roles is responsible for their biosynthesis. The major enzymes that synthesize chondroitin/dermatan sulfate are chondroitin synthase, glucuronyltransferases, sulfotransferases and dermatan sulfate epimerase. The study of these enzymes is of great interest as it seems that glycosaminoglycans undergo qualitative and quantitative changes in various types of cancer contributing to their development.

Statins belong to a group of pharmaceuticals that work by blocking the activity of HMG-CoA reductase, i.e., the conversion of HMG-CoA to mevalonic acid, which is the first step of the cholesterol biosynthesis. Apart from this property, their action on various signaling pathways is of great interest. Through research it seems that their action, mainly of lipophilic statins, helps to reduce the proliferation, signaling, growth and metastasis of cancer cells.

The present study focused on the examination of the expression of the synthases CHPF (chondroitin polymerizing factor), CHSY1 (chondroitin synthase-1), CHSY3 (chondroitin synthase-3), CHST3 (chondroitin 6-O-Sulfotransferase), CSGcAT (chondroitin sulfate glucuronyltransferase) and DSE (dermatan sulfate epimerase) in the presence of a lipophilic statin, simvastatin.

Our results showed that all enzymes were expressed in higher amounts in MDA-MB-231 than MCF-7 cells. In the presence of simvastatin, CSGcAT did not show significantly altered expression, CHPF remained almost constant in MDA-MB-231 cells, whereas it showed an almost dose-dependent increase in MCF-7 cells, and CHSY1 showed decreased expression at intermediate concentrations in both cells. The results suggested that simvastatin did not affect significantly the chondroitin synthases, except CHPF in MCF-7 cells, possibly leading to a more aggressive phenotype of these cells.

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Lack of LBR and Lamin A cause nuclear aberrations

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Lamin B Receptor (LBR) is a ubiquitous integral protein of the inner nuclear membrane (INM). It is involved in various important cellular activities, such as nuclear assembly, tethering of the nuclear lamina to the INM, chromatin remodeling and transcription regulation. There is growing evidence that LBR and Lamin A, a key component of the nuclear lamina, are critical players in “chromatin inversion”. During differentiation, many cell types do not express either Lamin A or Lamin A and LBR. In the later case, heterochromatin is dissociated from the INM and assembles in one major focus in the nuclear interior. A similar event is also observed when LBR is mutated in pathological conditions, such as Pelger-Huet anomaly in humans and ichthyosis in mice.

We are investigating LBR’s role in anchoring peripheral heterochromatin to the nuclear envelope and in chromatin rearrangements by examining its association with specific genomic loci and its cooperation and complementarity with other nuclear structures. To this end, we have generated stable NIH/3T3 cell lines that do not express LBR, Lamin A, or both. Only minor changes in the nuclear envelope (NE) and chromatin architecture have been detected in single (LBR or Lamin A) knockout cells. However, several defects in the growth rate, the architecture of the nuclear envelope and the localization of major INM components have been observed in double null mutants. In addition, chromatin distribution and localization of several chromatin markers seem to be considerably affected. Despite these effects, the double null cells proliferate and survive. In view of these findings, it is highly likely that there is a compensatory mechanism in place probably involving protein components of the NE and/or other nuclear structures.

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The oncoprotein SET/I2PP2A interacts with the histone deacetylase complex subunit SAP18 and modulates Sonic hedgehog signaling

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The hedgehog (Hh) signaling pathway is critical for the development of nearly every tissue and organ in vertebrates. Lack of Hh signaling during development is embryonic lethal, while hyperactivation of the Hh pathway promotes tumor formation and maintenance of a wide range of human malignancies, including medulloblastoma and basal cell carcinoma of the skin. Therefore, Hh signal transduction has emerged as a key pathway for cancer therapy and its pharmacological modulation is of great importance in clinical trials. Unlike other signaling pathways involved in development, the vertebrate Hh signaling is dependent on the primary cilium, a microtubule-based membrane protrusion that functions as an antenna in the cells. Gli transcription factors (Gli1, Gli2, Gli3) are the main effectors of the pathway and their activity controls both the amplitude and the outcome of the Hh expression program especially during development.

The oncoprotein SET/I2PP2A is a nuclear protein that functions as a histone chaperone, transcription cofactor and as an epigenetic regulator. Several studies have focused on SET targeting as a potential therapeutic approach in cancer, by testing the effects of SET inhibitors on cancer progression. In this study we show that the oncoprotein SET/I2PP2A is involved in the regulation of Gli-mediated transcription. We used gene transcription assays and CRISPR/Cas9-mediated targeting of set gene in zebrafish and cultured cells to show its involvement in Hedgehog signaling. Protein-protein interaction analysis revealed an interaction of SET/I2PP2A with the histone deacetylase complex subunit SAP18 (Sin3A-associated protein 18) which affects the transcriptional activity of Gli1 transcription factor and fine tunes the outcome of the hedgehog expression program during development.

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Clinical assessment of m6A RNA methylation machinery in childhood acute lymphoblastic leukemia

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Childhood acute lymphoblastic leukemia (chALL) represents the most prevalent pediatric cancer, accounting for approximately 26% of all childhood malignancies up to the age of 15 years worldwide. Despite the high 5-year survival rate, drug resistance and, subsequently, recurrence affect a substantial number of children, highlighting the clinical need in ameliorating disease management. N6-methyladenosine (m6A) constitutes the most abundant modification of eukaryotic RNAs and is introduced by a multicomponent complex consisting of the SAM-dependent METTL3 methyltransferase and accessory subunits (METTL14, WTAP, VIRMA and RBM15B). Although there is evidence for a link between m6A modifications and chALL, research on the subject is limited, while their potential clinical role has not been deciphered. Therefore, the aim of the present study has been to clinically evaluate the core complex of m6A RNA imprinting in chALL. To achieve our goal, we quantified the expression of m6A RNA methylation machinery by RT-qPCR in normal (n=23) and chALL (n=47) samples. Patients' death and disease relapse were used as clinical endpoints for survival analysis. Our findings revealed that METTL3 levels were downregulated in chALL patients compared to normal controls (p=0.045), while RBM15B levels were decreased in poor prednisone responders (p=0.010). Interestingly, loss of METTL3 (log-rank p=0.046 and HR=4.356; 95% CI: 0.902-21.04, p=0.067) and RBM15B (log-rank p=0.042 and HR=4.430; 95% CI: 0.919-21.36, p=0.064) were correlated with shorter overall survival. Combined clinical assessment unveiled the statistically significant association of loss of both METTL3 and RBM15B with worse survival outcomes (log-rank p=0.001 and HR=7.519; 95% CI: 1.876-30.13, p=0.004), independently of clinical and therapeutic response data (HR=30.795; 95% CI: 1.092-868.1, p=0.044). Conclusively, reduced METTL3 and RBM15B levels were linked to aggressive disease phenotype and dismal prognosis, suggesting that their evaluation could ameliorate chALL clinical management.

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NMR study of the interaction between the RRM2 of the human La protein and the IRES domain of Hepatitis C Virus

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La protein plays a key role in processing primary transcripts, including tRNAs, by facilitating proper folding. Although La seems to recognize and bind the 3' poly(U) tails of many artificial small RNAs, this feature is under debate for the natural ligands of La, and the exact mechanism is still unknown. Although La is mainly located in the nucleus, it can also be found in the cytoplasm, where it affects the translation of some cellular and viral mRNAs. Human La contains four distinct domains, namely La motif (LaM), two RNA recognition motifs (RRM1 and RRM2) and a variable C-terminus region. The La's C-terminus domain recognizes the Internal Ribosome Entry Site (IRES) of viral mRNAs and promotes their translation. Until today, the role of RRM2 and the mechanism of the IRES recognition by the La protein remain elusive.

Here, we present the binding study of the RRM2 domain on the HCV-IRES recognition. We performed NMR titration experiments, titrating polypeptides of the human La that contain the RRM2 domain with the IV domain of HCV-IRES. Because we wanted to study the binding properties of RRM2 domain and a possible role of the C-terminal region on La protein – HCV IRES recognition, we performed NMR titration experiments titrating polypeptides of human La protein that contain the RRM2 domain with the IV domain of HCV IRES. With a view to identifying the specific interaction sites of the RRM2-SBM (La 224-359), the RRM2-Cter (La 224-408), the RRM1-RRM2-SBM (La 105-359) and the RRM1-RRM2-Cter (La 105-408), increasing amounts of the unlabeled HCV-IRES were added to the ¹⁵N-labeled polypeptides. ¹H-¹⁵N HSQC spectra were recorded after each addition to monitor the chemical shift changes of the NH resonances. The interaction among the same La's polypeptides with the HCV-IRES were studied with ITC (Isothermal Titration Calorimetry) in parallel with NMR.

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Identification and characterization of a splice variant of human poly(A)-specific ribonuclease

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Poly(A)-specific ribonuclease (PARN) is a deadenylase that catalyses the shortening of poly(A) tails, which is the first and rate-limiting step in mRNA degradation. In addition, PARN mediates the maturation of a diverse and ever-expanding repertoire of non-coding RNAs. Multiple splice variants of PARN have been reported in RNA sequencing studies. Yet, a study on the biochemical characterization or biological significance of the enzyme's splice variants is still pending. Herein, we detect a splice variant of PARN, PARN_v1, in pleural malignant mesothelioma (PMM) cell lines. PARN_v1 shows increased mRNA and protein levels in cells originating from all PMM subtypes, whilst it is barely detectable in benign pleural cells. Cloning of PARN_v1 mRNA from M14K PMM cells and sequence analysis revealed that the variant results from an alternative 5' splice site selection at the 3' end of exon 1. The omitted sequence includes the original start codon of full-length PARN and translation starts at a downstream start codon located at exon 4. Compared to the full-length PARN, the resulting PARN_v1 polypeptide lacks a 61-amino acid sequence from its N-terminus including two catalytic residues. Surprisingly, deadenylation assays using labelled 3'-oligoadenylated substrates reveal that PARN_v1 retains its poly(A)-shortening activity. Molecular modelling and site-directed mutagenesis reveals residues that shape the active site of PARN_v1 and compensate for the loss of the abovementioned catalytic ones of full-length PARN. To identify factors that may regulate the levels of PARN and PARN_v1, we investigate the role of several microRNAs in PMM cell lines. Given the role of PARN in the regulation of gene expression the investigation of the biological significance of its splice variants remains an open challenge.

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Novel circular transcripts (circRNAs) of the human *BAX* gene discovered using targeted third-generation sequencing, based on the nanopore technology

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Circular RNAs (circRNAs) are circular transcripts of genes deriving from back-splicing events. Their functions are multiple, as they may act as sponges, scaffolds, or transportation vehicles, as well as regulators of transcription and protein-coding transcripts. The elucidation of their regulatory potential has revealed their involvement both in normal and pathological processes, such as cancer. The BCL2-associated X protein (BAX), is a significant mediator of the mitochondrial apoptotic pathway, inducing the permeabilization of the mitochondrial membrane and the consequent release of cytochrome c into the cytosol. Taking into consideration the biological importance of BAX, we aimed to discover novel circular RNA transcripts of *BAX* in human breast cell lines. In order to achieve this, total RNA was extracted from eleven breast cancer cell lines and one normal cell line. Reverse transcription followed, using random hexamers and the produced cDNA was used for first- and second-round PCR. PCRs were performed using *BAX*-specific divergent primers, resulting in the amplification of circular transcripts from the *BAX* gene. The PCR products were sequenced, using nanopore technology, in the MinION Mk1C sequencer with the Flongle adapter. Finally, bioinformatic analysis was conducted using publicly available tools, as well as *in-house* developed algorithms to align reads, manipulate alignments, annotate, and find transcripts. The aforementioned workflow, revealed novel circular RNAs of *BAX*, comprising of different combinations of exons and with specific exonic regions being more abundant than others. Moreover, both truncations and extensions of the known exons can be observed in the circular transcripts. Furthermore, in some circRNAs, bases next to back-splicing sites may derive from both regions which contribute to the back-splice junction. Finally, the presence of most circular transcripts seems to be uneven between the breast cell lines which were used, indicating a distinct molecular profile of the human breast cell lines.

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Expression, purification and antigenic characterization of the West Nile Virus capsid protein

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West Nile Virus (WNV) is a member of the Flaviviridae family that can occasionally cause severe neuroinvasive disease. The viral genome is a positive-sense ssRNA molecule and encodes 7 non-structural and 3 structural proteins. The structural proteins consist of the pre-membrane (preM), the envelope (E) and the capsid (C) protein and participate in the formation of an icosahedral enveloped virion. Of these, E protein generates high levels of neutralizing antibodies, and it is used, alone or in combination with the preM for the development of serological assays. In contrast, the antigenic properties of C protein as well as its role in WNV serological diagnostics remain elusive. As current WNV serological tests have limitations due to cross-reactivity with other flaviviruses, we sought to investigate the ability of the viral capsid protein to induce antibodies in WNV infected individuals.

To this end, the capsid protein from the WNV strain NY99 was synthesized in both prokaryotic and eukaryotic expression systems based on the Rosetta(DE3)pLysS E.coli strain combined with the pET-28a(+) vector or the Sf9 insect cells combined with Bac-to-Bac baculovirus vector respectively. Expression vectors in both systems introduced a His-tag in the N-terminus of the recombinant capsid proteins which facilitates protein detection as well as protein purification using Ni-NTA affinity chromatography. The purified recombinant core protein(s) were used in immunoblot assays to test the presence of anti-core antibodies in the serum of WNV infected individuals. Interestingly, both recombinant C proteins are recognized by antibodies in the tested sera. Specifically the Sf9-produced C protein in 7 out of 10 sera whereas the E.coli-produced C protein in 9 out of 10 sera. Our results suggest that the WNV core protein is a promising candidate antigen for the development of improved WNV serological assays. Currently, we are investigating further the clinical value of our methodology.

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Structural studies of the SARS Unique Domain (SUD) of SARS-CoV-2 with its' intracellular partner, the human protein RCHY1, through biophysical studies

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Severe acute respiratory syndrome coronavirus 2 (SARS CoV-2) is a highly pathogenic virus, cause of the worldwide pandemic COVID-19. Its positive sense, single stranded RNA encodes, among others, 16 non-structural proteins (nsPs), which form a replication–transcription complex (RTC) vital for the virus's proliferation inside the host.¹ NsP3, the largest one, possesses multiple domains, including the SARS Unique Domains (SUDs). The N-terminal, middle, and C-terminal domains—abbreviated SUD-N, SUD-M, and SUD-C—are its three subdomains. The last domain adopts a frataxin-like fold, while the first two, a macro-like fold.² Considering that SUDs of SARS CoV and SARS CoV-2 share a significant degree of amino acid sequence identity, it is assumed, that they will also share similar functions, in terms of participation in viral replication and interactions with the host's proteins. These include the ability, of SUD-N and -M domains, to bind guanine-rich, non-canonical nucleic acid structures known as G-quadruplexes and the regulation of the activity of the E3 ligase ring-finger and CHY zinc-finger domain-containing 1 protein (RCHY1).

RCHY1's most crucial regulator is the antiviral protein p53, while RCHY1 acts as a negative regulator of this factor, especially its transcriptionally active form. Therefore, increased levels of p53 lead to its poly-ubiquitination and, consequently, to its proteolytic degradation. Besides, interaction between RCHY1 and the SUDs of SARS-CoV stabilize the E3 ligase, which induces further the poly-ubiquitination of endogenous p53. The SUD domains bind to RCHY1 at a region between residues 95 and 144, while both N and M domains are required for the interaction.³ Nevertheless, the interaction between the SUDs of SARS CoV-2 and RCHY1 has not been clarified yet and it needs further investigation. Herein we use NMR to monitor titration experiments and to clarify the elucidation of the interaction between SUD-NM and RCHY1.

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References

- 1 Gallo, A. et al. ¹H, ¹³C and ¹⁵N chemical shift assignments of the SUD domains of SARS-CoV-2 non-structural protein 3c: "the N-terminal domain-SUD-N". *Biomol NMR Assign* 15, 85–89 (2021).
- 2 Lavigne, M. et al. SARS-CoV-2 Nsp3 unique domain SUD interacts with guanine quadruplexes and G4-ligands inhibit this interaction. *Nucleic Acids Res* 49, 7695–7712 (2021).
- 3 Ma-Lauer, Y. et al. P53 down-regulates SARS coronavirus replication and is targeted by the SARS-unique domain and PLpro via E3 ubiquitin ligase RCHY1. *Proc Natl Acad Sci U S A* 113, E5192–E5201 (2016).

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Biophysical characterization of an unexplored viral macro domain: Rubella macro domain

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Almost three decades ago, bioinformatics approaches identified a unique conserved gene domain within four viral families, the Togaviridae, Matonaviridae, Coronaviridae, and Hepeviridae. All these families are positive-sense RNA viruses and cause disease in humans and animals. The domain was named the 'X' domain as its structure and function was unknown. These domains were defined as 'macro domains' after crystal structures of both cellular and viral macrodomains revealed a core fold homologous to the nonhistone part of the macroH2A protein. Most of these macro domains were shown to have the ability to hydrolytically remove single ADPr units from ADP-ribosylated substrates, a function known as De-Marylation, which is important for the proliferation of the viruses.

Rubella virus, which is a member of the Matonaviridae family, causes a congenital syndrome characterized by multiorgan birth defects. However, this disease has largely disappeared in developed countries since an effective vaccine was developed, but in some regions this threat still exists, thus is necessary to also develop drugs against it. A target that is very interesting for its druggability is the macro domain of Rubella, which domain is understudied, and our knowledge is limited. Therefore, the conformational dynamics, the interaction with partners or small molecules and the biochemical properties of the macro domain of Rubella is studied herein by NMR spectroscopy, ITC, and western blotting.^{1,2}

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References

- 1 A. R. Fehr, G. Jankevicius, I. Ahel, and S. Perlman, "Viral Macrodomains: Unique Mediators of Viral Replication and Pathogenesis," *Trends in Microbiology*, vol. 26, no. 7. Elsevier Ltd, pp. 598–610, Jul. 01, 2018. doi: 10.1016/j.tim.2017.11.011.
- 2 A. C. Tsika et al., "Binding adaptation of GS-441524 diversifies macro domains and downregulate SARS-CoV-2 de-MARylation capacity," *J Mol Biol*, p. 167720, Jul. 2022, doi: 10.1016/j.jmb.2022.167720.

P204

Structural and functional studies of viral methyltransferase of Hepatitis E Virus

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Hepatitis E virus (HEV) is identified as the most common cause of acute viral hepatitis worldwide. HEV infection is estimated to cause 70,000 deaths from acute liver failure. There are seven genotypes (HEV1-HEV7), yet only the first four genotypes are responsible for human infection. According to the statistical incidence data, 20 million cases of HEV infection occur annually, while approximately 3.3 million result in symptomatic infections. Transmission occurs mainly through the fecal-oral route, but also through contaminated blood transfusions, transplants, and from mother to embryo [1]. HEV belongs to the Hepeviridae family and its genome is a 7.2Kb ss(+) RNA. It consists of a 5' untranslated region (5'-UTR), 3 open reading frames (ORF's) and a 3' untranslated region(3'-UTR) which ends in a polyadenine tail [2]. Each ORF has a crucial role for a successful viral transmission and human infection.

Our study attempts to elucidate the physicochemical and structural properties of two components of the ORF-1, which are necessary for RNA replication. These functional domains are the methyltransferase (vMT) and the macro domain (vMD). HEV vMT catalyzes the transfer of the methyl group from S-adenosylmethionine to GTP, resulting in m7GTP, a procedure known as mRNA capping. mRNA capping is essential for virus life cycle and the multiplication process [3]. Additionally, vMD serves not only a central role in a variety of cellular activities, including de-MARylation and de-PARylation, but also may be associated with persistent infection through the interaction with MT [4]. In the present study, we describe the cloning, expression, purification of vMT protein of HEV and the direct interference with the de-Marylation activity of vMD. Specifically, a variety of experiment conditions were tested for the expression of different recombinant polypeptides of vMT, to find the appropriate polypeptide for the structural and the protein-protein interaction studies through NMR spectroscopy.

References

1. N. Kamar et al., "Hepatitis E virus infection," *Nat Rev Dis Primers*, vol. 3, no. 1, p. 17086, Dec. 2017
2. P. Kar et al., "A Review of the Diagnosis and Management of Hepatitis E," *Curr Treat Options Infect Dis*, vol. 12, no. 3, pp. 310–320, Sep. 2020
3. E. Decroly et al., "Conventional and unconventional mechanisms for capping viral mRNA," *Nat Rev Microbiol*, vol. 10, no. 1, pp. 51–65, Jan. 2012
4. S. Anang et al., "Identification of critical residues in Hepatitis E virus macro domain involved in its interaction with viral methyltransferase and ORF3 proteins," *Sci Rep*, vol. 6, no. 1, p. 25133, Jul. 2016

Acknowledgments: We acknowledge support of this work by the project "INSPIRED-The National Research Infrastructures on Integrated Structural Biology, Drug Screening Efforts and Drug target functional characterization" (MIS 5002550) which is implemented under the Action "Reinforcement of the Research and Innovation Infrastructure", funded by the Operational Programme "Competitiveness, Entrepreneurship and Innovation" (NSRF 2014-2020) and co-financed by Greece and the European Union (European Regional Development Fund).

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The study of the effect of ligand *m*-nitrophenol on the molecular and crystalline polymorphism of Human Insulin

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This study focuses on the experimental procedure followed to determine human insulin (HI) polymorphism, after its co-crystallization with the organic molecule 3-nitrophenol (*m*-nitrophenol) under different *pH* conditions (*pH* 4.10 to 8.50). For this purpose, solutions of insulin hexamers, obtained and stabilized in the presence of ions, and the possible organic ligand were prepared and let for crystal growth, via the batch crystallization method. X-ray Powder Diffraction (XRPD) was utilized to determine, crystallographically, the resulting polycrystalline protein samples. Experiments were conducted in the facilities at the Department of Biology in the University of Patras (Biochemistry, Structural Biology and Crystallography lab, Laboratory Y31) using the Malvern Panalytical X'Pert PRO laboratory diffractometer, as well as the European Synchrotron Radiation Facility (ESRF) on beamline ID22. Analysis of the diffraction data occurred at the level of unit-cell symmetry and led to the identification of three distinct crystal polymorphs of monoclinic (space group $P2_{1(e)}$ with the following lattice parameters: $a = 72.537 \text{ \AA}$, $b = 64.069 \text{ \AA}$, $c = 59.614 \text{ \AA}$, $\beta = 92.018^\circ$ and $V = 2.7688 \cdot 10^5 \text{ \AA}^3$), rhombohedral (space group $R\bar{3}$ with the following lattice parameters: $a = b = 79.856 \text{ \AA}$, $c = 40.254 \text{ \AA}$ and $V = 2.2228 \cdot 10^5 \text{ \AA}^3$), and cubic symmetry (space group $I213$ with the following lattice parameters: $a = b = c = 78.867 \text{ \AA}$ and $V = 4.90570 \cdot 10^5 \text{ \AA}^3$), in the slightly acidic, slightly alkaline and alkaline *pH* ranges, respectively. The new crystalline phase of monoclinic symmetry presents interesting characteristics for the future development of microcrystalline insulin drugs. In addition to the X-ray Powder Diffraction (XRPD) experiments, Single Crystal X-ray Diffraction (SCXRD) experiments were carried out to enhance the reliability of the results.

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Structural determination of the Phe964Ala Arkadia mutant via NMR Spectroscopy

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Ubiquitination is a post-translational modification responsible for a variety of cellular functions including DNA repair, apoptosis and protein degradation. Initially, ubiquitin is activated by the E1 enzyme in an ATP-dependent manner which results in the formation of a thioester bond between the glycine-76 of ubiquitin and a cysteine of the E1. Afterwards, the activated ubiquitin is transferred to the catalytic cysteine of the E2 conjugating enzyme. In the final step, the E3 ligase facilitates the transmission of ubiquitin to the substrate. The target protein may undergo several types of ubiquitination each of which results in diverse outcomes¹.

Arkadia is an E3 ubiquitin ligase that positively regulates the transforming growth factor- β (TGF- β) pathway by promoting ubiquitin-dependent degradation of the negative regulators Smad7, c-Ski and SnoN. Arkadia possesses a RING-H2 domain at its C-terminus, which exhibits a canonical RING $\beta\beta\alpha$ topology consistent to other RING domains. The 3-turn α -helix and the two zinc-binding loops of Arkadia RING domain play a crucial role in the interaction with the E2 enzymes². We have previously demonstrated that mutations in conserved residues of the RING domain result in structural alterations of the domain and therefore its interaction with the E2 enzyme³. In this study we investigate the pivotal role of Phe964 of Arkadia, a sequentially and structurally conserved residue in many RING domains, via NMR spectroscopy. This residue is located at the hydrophobic core of the protein and is considered a "linker" between the second and third binding motif. Phe964Arg mutant led the protein to an unfolded state, contrary to Phe964Ala mutant that was well-folded. Phe964Ala mutant displayed differences in the signal dispersion compared with the wild type one and exhibited no interaction with E2 UbcH5b. In order to estimate the impact of this mutation to the RING domain, structural, dynamical and functional characterization was conducted.

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References

- ¹ Pickart CM.et al. (2004). Ubiquitin: structures, functions, mechanisms. *Biochimica et Biophysica Acta* 1695, 55-72
- ² Chasapis CT.et al. (2012). NMR-based insights into the conformational and interaction properties of Arkadia RINGH2 E3 Ub ligase. *Proteins* 80(5), 1484-9
- ³ Birkou M.et al. (2017). A residue specific insight into the Arkadia E3 ubiquitin ligase activity and conformational plasticity. *Journal of Molecular Biology* 429, 2373-2386.

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The T₂ structure of polycrystalline cubic human insulin

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The polymorphism of human insulin upon pH variation was characterized via X-ray powder diffraction, employing a crystallization protocol previously established for co-crystallization with phenolic derivatives. Two distinct rhombohedral (R3) and one cubic (I2₁3) polymorphs were identified with increasing pH, corresponding to the T₆, T₃R₃^f and T₂ conformations of insulin, respectively. The structure of the cubic T₂ polymorph was determined via a multi-profile and stereochemically-restrained Rietveld refinement at 2.7 Å resolution. This constitutes the first cubic insulin structure determined from crystals grown in the presence of zinc ions, although no zinc binding was observed. Differences of the polycrystalline variant to other cubic insulin structures, as well as the nature of the pH-driven phase transitions are discussed in detail.

These authors contributed equally (D. P. Triandafillidis, Maria Athanasiadou).

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Structural and biophysical insights into Hepatitis E virus macro domain

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Hepatitis E virus (HEV) is an emerging pathogen causing 20 million infections worldwide, leading to an estimated 3.3 million symptomatic cases with ~56,600/year being lethal. HEV belongs to the Hepeviridae family among the most broadly known types of Hepatitis such as A, B, C and D. In developing countries, HEV is spread by the fecal–oral route, while in developed countries the routes of transmission include the ingestion of undercooked meat or meat products derived from infected animals' transfusion of infected blood products and vertical transmission from a pregnant woman to the embryo. The genome of HEV is ~7.2 kb ss(+)RNA with a 5' 7-methylguanosine cap structure followed by a short 5' untranslated region (UTR), three major open reading frames ([ORFs]: ORF1, ORF2, and ORF3), and a 3'UTR¹.

This study focus on the structural and biophysical characterization of the macro domain, which is encoded in the ORF1. In general, viral MDs (vMD) can bind both ADP-ribose and its derivatives and the possess a key-role in the recognition and removal of ADP-ribosylation. Specifically, vMDs have been identified as erasers of a single or several ADP-ribose moieties (de-MARylation/de-PARylation, respectively) and therefore may be implicated in mechanisms developed by the viruses to escape the early stages of host-immune response². In the present study, a wide range of experimental conditions were tested for the expression of different length recombinant polypeptides of vMD and achieved the identification of a polypeptide that renders stability to the protein domain. Also, to gain insights into the biophysical properties of the vMD we performed interaction studies with ADPr and other adenosine derivatives through heteronuclear NMR Spectroscopy and Isothermal Titration Calorimetry (ITC).

References

- ¹ Debing, Yannick, et al. "Update on hepatitis E virology: Implications for clinical practice." *Journal of hepatology* 65.1 (2016): 200-212.
- ² Wißing, Michael H., et al. "Virus–Host Cell Interplay during Hepatitis E Virus Infection." *Trends in Microbiology* (2020).

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Physicochemical and spectroscopical study of bacterial homologs of $\beta 1$ H-NOX domain of human soluble guanylyl cyclase (sGC)

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Heme-Nitric oxide/Oxygen binding (H-NOX) domains are a family of gas-sensing hemoproteins that bind diatomic gases. In humans, H-NOX domains are part of soluble Guanylate Cyclase (sGC) and function as a sensor of NO, resulting in a series of conformational and charge changes which lead to the enzymatic activation of sGC. sGC is a heterodimer enzyme composed of two subunits, alpha and beta. H-NOX domain, which is located at the N-terminus of the regulatory $\beta 1$ subunit of sGC and bears a heme molecule, seems to be crucial in catalysis of conversion of GTP (guanosine 5'-triphosphate) to cGMP (cyclic guanosine 3,5 -monophosphate) to this effect downstream changes in cellular homeostasis. The NO/sGC/cGMP signaling pathway is dysfunctional in many diseases, such as cardiovascular diseases. In that case, two categories of chemical compounds named sGC "activators" and "stimulators" aim to restore and enhance enzyme's activity [1]. H-NOX domain is conserved in eukaryotes and bacteria and consists of about 190 amino acids. Fe-heme as a substrate can bind diatomic gases, such as NO, CO and O₂ with different binding affinities [2]. As mentioned, H-NOX domains initiate signaling cascades on binding gaseous ligands or chemical compounds, but how this is accomplished remains unclear. In this direction, we examine three bacterial H-NOX proteins from *Nostoc punctiforme* (Npun H-NOX), *Shewanella oneidensis* (So H-NOX) and *Shewanella woodyi* (Sw H-NOX). These bacterial domains, because of high amino acid identity, may explain to an extent the organism-specific ligand preference and how the redox state of heme determines H-NOX active site and coordination upon binding of diatomic gases. Nuclear magnetic resonance (NMR) can give us information about the structural changes in common key-regions of the selected H-NOX domains that will help us to understand the mechanism of enzyme activation.

References

¹ Argyriou, A.I. et al. (2021), *CRSB*, 3, pp. 324–336.

² Chasapi, S.A., Argyriou, A.I. and Spyroulias, G.A. (2022), *Biomol NMR Assign.*

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E2 conjugating enzyme selectivity and enzymatic activity of E3 ligase Arkadia

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Ubiquitination is a fundamental post-translational modification that regulates a host of cellular functions, mainly by mediating the selective proteasomal degradation of regulatory proteins. The process is catalyzed by the concerted action of activating (E1), conjugating (E2) and ligating (E3) enzymes and its deregulation has been implicated as a causative factor in various diseases, including cancer. Arkadia/RNF111 is a RING E3 ubiquitin ligase that enhances the transforming growth factor- β (TGF- β) signaling responses, through ubiquitin-dependent degradation of negative regulators of the pathway i.e., the inhibitory SMAD7, transcriptional co-repressor SKI and its close homologue SNON (SKIL)¹. Arkadia is also classified as a SUMO-targeted ubiquitin ligase (STUbL) implicated in the DNA damage response pathway. Specifically, Arkadia interacts with heterodimer UbcH13/MMS2 E2 complex to promote the formation of K63-linked ubiquitin chains to SUMOylated proteins e.g., Xeroderma Pigmentosum group C (XPC)² and Promyelocytic Leukaemia protein (PML)³.

Despite the pivotal role of Arkadia in many pathways, little is known about the features that modulate its enzymatic function. We have previously shown that, unlike most other E3 ligases, RING domain of Arkadia is not fully active and requires external elements to activate an E2~Ub complex⁴. This study has the potential to provide understanding on how Arkadia recognizes its physiological E2 partners and whether E2-Arkadia interaction leads to a productive complex. The interaction of Arkadia with the E2 partners UbcH5B, UbcH13, as well as UbcH7, is studied through NMR Spectroscopy. In this context we demonstrate that the interfaces of Arkadia-UbcH5B and Arkadia-UbcH13 complexes remain constant in both cases. In contrast, Arkadia-UbcH7 complex displays a relatively limited interaction surface in agreement with its inability to perform ubiquitination. Furthermore, we revealed that the synergistic action of Arkadia with different E2s, catalyzes either mono- or poly-ubiquitin chain formation, that can lead to distinct biological outcomes.

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References

- ¹ Nagano, Y. et al. Arkadia induces degradation of SnoN and c-Ski to enhance transforming growth factor- β signaling. *JBC* 282, 20492–20501 (2007).
- ² Poulsen, S. L. et al. RNF111/Arkadia is a SUMO-targeted ubiquitin ligase that facilitates the DNA damage response. *J. Cell Biol.* 201, 787–807 (2013).
- ³ Erker, Y. et al. Arkadia, a Novel SUMO-Targeted Ubiquitin Ligase Involved in PML Degradation. *Mol Cell Biol* 33, 2163–2177 (2013).
- ⁴ Birkou, M. et al. Unveiling the Essential Role of Arkadia's Non-RING Elements in the Ubiquitination Process. *Int J Mol Sci* 23, 10585 (2022).

P211

Use of Structure-Based Virtual Screening for the identification of novel allosteric inhibitors that target the Thioredoxin Reductase (TrxR) of *Escherichia coli*

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The thioredoxin (Trx) system, along with the glutathione based glutaredoxin system, channel the reducing antioxidant electron flow to the cytosol of most living cells including bacteria and viruses. The two systems may regulate a plethora of additional cellular processes such as DNA synthesis and repair, and the activation of redox-dependent transcription factors. In the Trx system, electrons are sequentially transferred from NADPH to thioredoxin reductase (TrxR), Trx and finally to the substrates of the latter. Differences in the structure and catalytic mechanisms between mammalian and bacterial TrxRs render the bacterial enzymes as potential drug targets. In the present study, Structure-Based Virtual Screening (SBVS) was performed for the discovery of novel allosteric inhibitors that may selectively target the TrxR of *Escherichia coli* (EcTrxR). To achieve maximum selectivity of the hit compounds, we used a crystal structure of the oxidized form of EcTrxR (PDB ID: 1TDE), where we implemented a straight-forward SBVS protocol. With the use of the MCULE online platform, molecular docking was performed with the Autodock Vina algorithm for the ~9 million compounds marked as "in stock" in the MCULE database. Based on the docking score, the 1000 top-ranking compounds were selected and subjected to toxicity and drug-likeness filters. Next, further validation was performed by comparing our results to the molecular docking properties of known inhibitors of EcTrxR with IC₅₀ values taken from the ChEMBL database (e.g., ebselen). Ten top hit compounds were finally selected after close inspection of their interactions with the residues of the active site (hydrogen bonding, salt bridges and van der Waals interactions). The selected compounds appear as good candidates for further investigation as antimicrobials.

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Molecular and crystalline polymorphism of human insulin in the presence of gallic acid

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The molecular and crystalline polymorphism of Human Insulin (HI) in the presence of the organic compound 3,4,5-trihydroxybenzoic acid (gallic acid) was studied via the combination of both X-Ray crystallography and Nuclear Magnetic Resonance (NMR). For this purpose, solutions of human insulin and gallic acid were subjected to Saturation Transfer Difference NMR (STD-NMR) experiments, which revealed a dissociation constant (K_d) equal to 154 μM that is characteristic of a binding affinity between these two molecules. To further detect the binding via X-ray Crystallography, a co-crystallization experiment of insulin in the presence of gallic acid was carried out to produce polycrystalline samples (powder samples), utilizing the batch method, under different pH conditions (4.21 to 8.01). The powder samples were measured via X-Ray Powder Diffraction (XRPD) experiments, using synchrotron radiation at the European Synchrotron Radiation Facility (ESRF) in Grenoble, France. The X-Ray diffraction data were analyzed in terms of a unit-cell employing the crystallographic package program HighScore Plus and they demonstrated the existence of three polymorphs. Under acidic and neutral pH conditions two crystalline phases with rhombohedral ($R\bar{3}$) symmetry were revealed, corresponding to the T_6 ($a=82.322(3)$ Å, $c=34.180(1)$ Å) and $T_3R^f_3$ ($a=80.692(2)$ Å, $c=38.673(2)$ Å) molecular conformation of insulin, whilst under alkaline conditions crystals obtained a cubic ($I2_13$) symmetry ($a=78.815(7)$ Å) with a T_2 stereochemical conformation. These polymorphs correspond to ligand-free insulin crystals, so the absence of a new polymorph indicates an uncommon binding motif of gallic acid.

Bibliography

- Karavassili, F., Valmas, A., Fili, S., Georgiou, C. D., & Margiolaki, I. (2017). In quest for improved drugs against diabetes: The added value of X-ray powder diffraction methods. *Biomolecules*, 7(3). <https://doi.org/10.3390/biom7030063>
- Smith, G. D., Pangborn, W. A., & Blessing, R. H. (2001). Biological Crystallography Phase changes in $T_3R^f_3$ human insulin: temperature or pressure induced? *Acta Cryst*, 57, 1091–1100.
- Smith, G. D., Pangborn, W. A., & Blessing, R. H. (2003). Biological Crystallography The structure of T_6 human insulin at 1.0 Å resolution.
- Spiliopoulou, M., Triandafillidis, D. P., Valmas, A., Kosinas, C., Fitch, A. N., von Dreele, R. B., & Margiolaki, I. (2020). Rietveld Refinement for Macromolecular Powder Diffraction. *Crystal Growth and Design*, 20(12), 8101–8123. <https://doi.org/10.1021/acs.cgd.0c00939>
- Spiliopoulou, M., Valmas, A., Triandafillidis, D. P., Fili, S., Christopoulou, M., Filopoulou, A. J., Piskopou, A., Papadea, P., Fitch, A. N., Beckers, D., Degen, T., Gozzo, F., Morin, M., Reinle-Schmitt, M. L., Karavassili, F., Rosmaraki, E., Chasapis, C. T., & Margiolaki, I. (2021). High-throughput macromolecular polymorph screening via an NMR and X-ray powder diffraction synergistic approach: The case of human insulin co-crystallized with resorcinol derivatives. *Journal of Applied Crystallography*, 54, 963–975. <https://doi.org/10.1107/S160057672100426X>
- Triandafillidis, D. P., Karavassili, F., Spiliopoulou, M., Valmas, A., Athanasiadou, M., Nikolaras, G., Fili, S., Kontou, P., Bowler, M. W., Chasapis, C. T., von Dreele, R. B., Fitch, A. N., & Margiolaki, I. (2022). The T_2 structure of polycrystalline cubic human insulin The T_2 structure of polycrystalline cubic human insulin. <http://journals.iucr.org/services/docxtemplate/> (Submitted)

P213**Heterochromatin Dynamics and Remodeling in Embryonic Stem Cells**

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Cell differentiation is associated with progressive immobilization of chromatin proteins, expansion of heterochromatin, decrease of global transcriptional activity and induction of lineage-specific genes. However, how these processes relate to one another remains unknown. We have found that the heterochromatic domains of mouse embryonic stem cells (ESCs) are dynamically distinct and possess a mosaic substructure. Although random spatio-temporal fluctuations reshuffle continuously the chromatin landscape, each heterochromatic territory maintains its dynamic profile, exhibiting robustness and resembling a quasi-steady state. Transitions towards less dynamic states are detected sporadically as ESCs downregulate Nanog and exit the self-renewal phase. These transitions increase in frequency after lineage-commitment, but evolve differently depending on cellular context and transcriptional status. We are now investigating whether chromatin remodeling relates to lineage selection and evolves in coordination to changes in the gene expression program.

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Determination of Endocrine Disruptors in Infant Formula and Baby Food Samples

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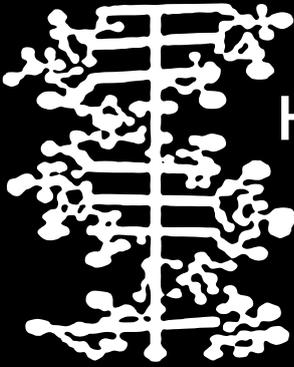
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Endocrine disruptors are environmental and food contaminants that pose a threat to human health, especially to vulnerable population, such as infants. The aim of this research is the determination of the levels of endocrine disruptors in commercially available infant formulas, baby milk and food. The substances studied include bisphenol A (BPA) and S (BPS), methyl (MeP) and propyl (ProP) parabens, HCB, DDT and its metabolites and PCBs (28, 52, 101, 118, 138, 153, 180). Samples were categorized by food type and processed by liquid-liquid extraction method. The analysis was held with Liquid Chromatography and Gas Chromatography combined with mass spectrometry systems (LC-MS and GC-MS). In infant formula and baby milk samples, BPA, ProP, HCB, PCB-28 and op-DDE were the most frequent detected substances, while PCB-28 and HCB were detected in almost all baby food samples. Finally, PCB-180 was not detected in infant formula and baby milk samples and PCB-153 and PCB-101 were not detected in any of the infant formula and baby food samples, respectively. After determining the concentration of the above substances in the samples, a risk assessment study was performed to assess the exposure of infants to them through diet. Risk assessment calculations were performed for infant formulas and median Hazard Index for bisphenols and parabens were less than 1, while median estimated daily intake (EDI) for the rest of the substances were also very low.



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