71ο Πανελλήνιο Συνέδριο ΕΛΛΗΝΙΚΗΣ ΕΤΑΙΡΕΙΑΣ ΒΙΟΧΗΜΕΙΑΣ και ΜΟΡΙΑΚΗΣ ΒΙΟΛΟΓΙΑΣ

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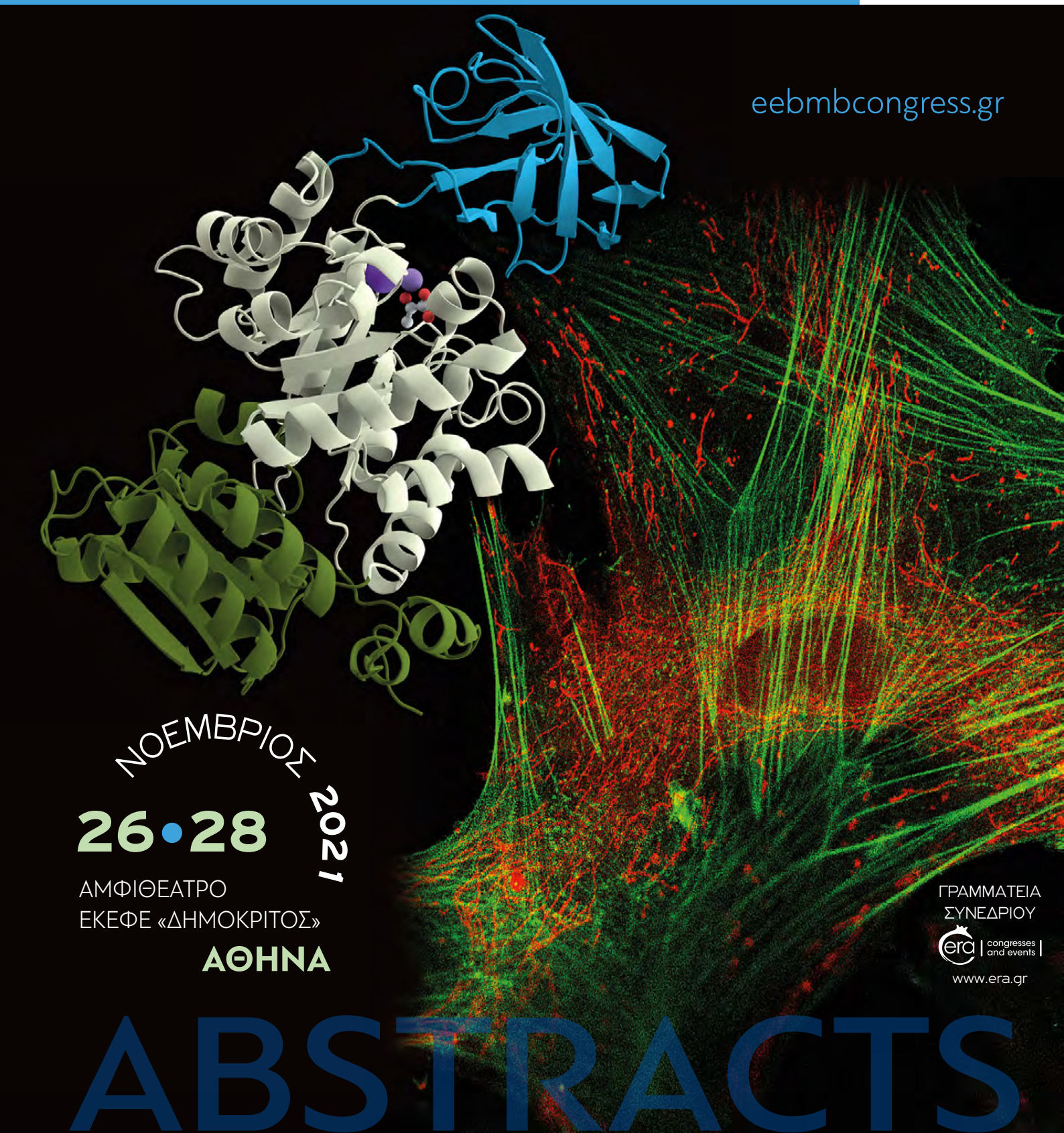

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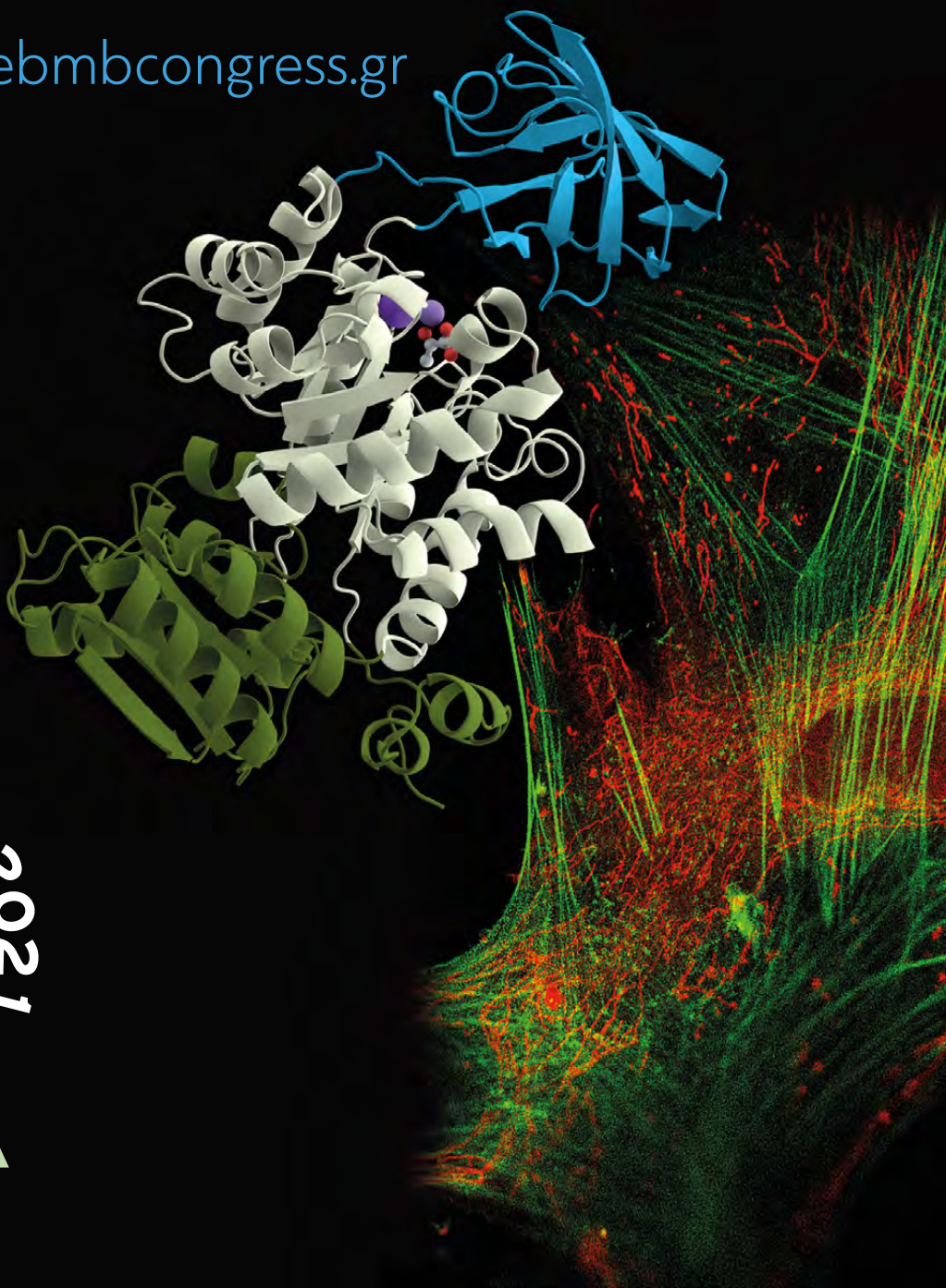

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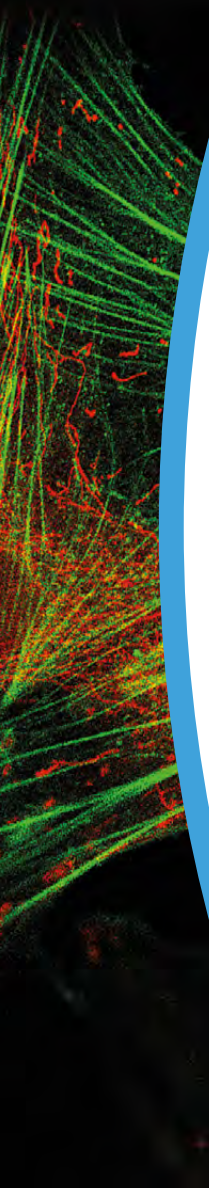

ABSTRACTS

INVITED LECTURES

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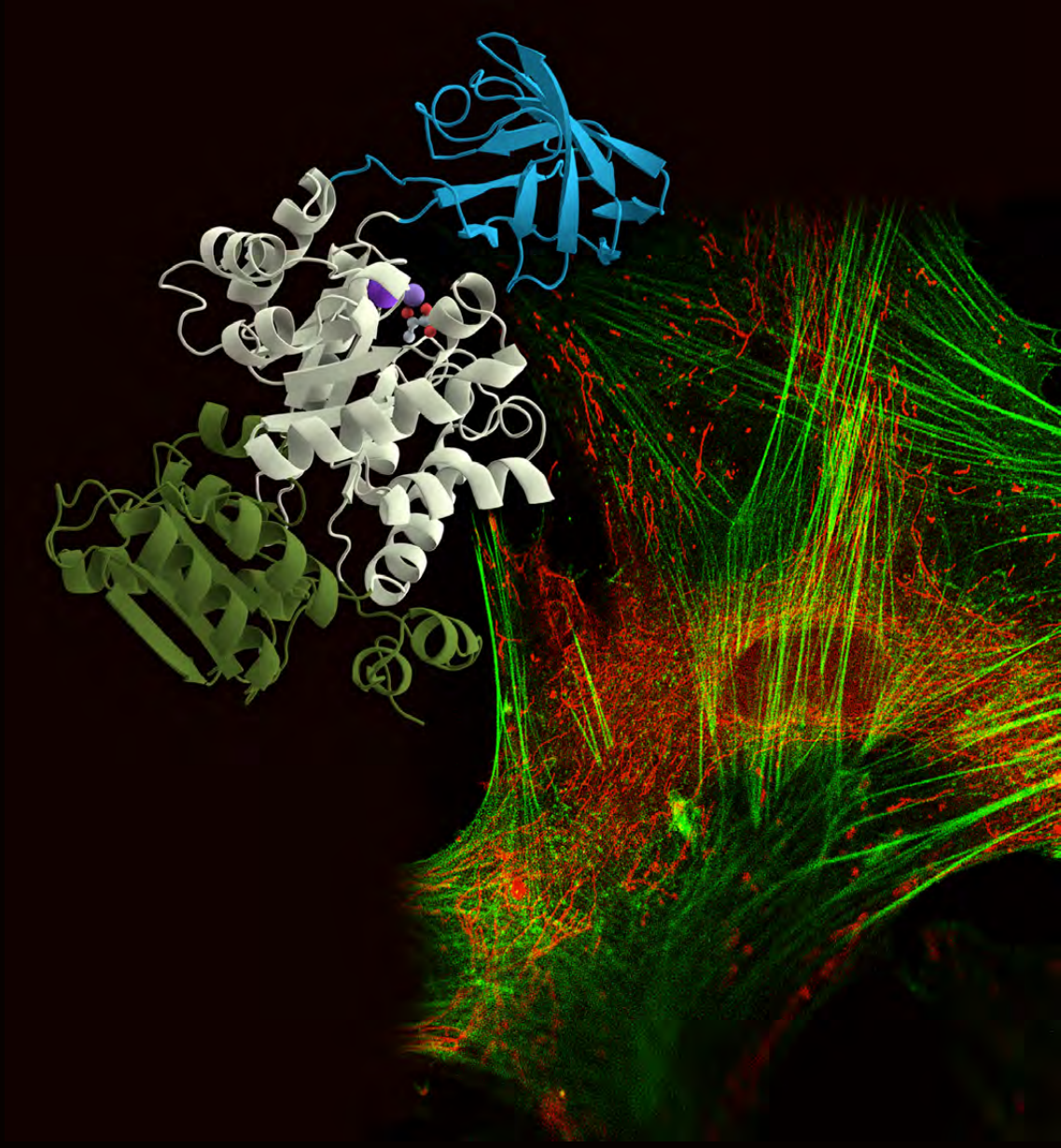

invited lectures_

71 Πανελλήνιο Συνέδριο
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Transcription-dependent and -independent pathways of the cellular response to hypoxia

Ilias Mylonis
Laboratory of Biochemistry, Faculty of Medicine, University of Thessaly, Larissa, Greece
**e-mail: mylonis@med.uth.gr*

Reduced oxygen availability (hypoxia) is a trait of various physiological and pathological conditions including ischemia and cancer. In order to adapt to hypoxia, cells rely upon the activation of the Hypoxia Inducible Factors (HIF), a small family of heterodimeric transcriptional activators. However, the cellular response to low oxygen conditions also involves, lesser-known, HIF- and transcription-independent processes that may occur very early after the onset of hypoxia. Our recent research includes the investigation of both pathways. Despite the existence of an oxygen sensing machinery that regulates the stability of the HIF-1α subunit, fine tuning of HIF-1 activity involves its direct phosphorylation. Investigation of HIF-1α phosphorylation by ERK1/2 revealed an intricate stimulatory mechanism that promotes HIF-1α nuclear accumulation and its association with NPM1, a histone chaperone that resides constitutively on HIF-1 target gene promoters and is essential for their activation and cancer cell survival under hypoxia. In parallel, recent results show that, shortly after the establishment of hypoxia, transcription-independent processes affect major constituents of the nuclear matrix and induce changes in both nuclear architecture and mRNA splicing.
Overall, our results suggest that the cellular response to oxygen deprivation is multilayered. It starts in the first minutes of hypoxia by remodeling nuclear structures, which may be important for reprogramming chromatin functions and mRNA processing, and continues with finely-tuned HIF-mediated gene expression, both important for cellular adaptation and survival under low oxygen conditions.

TORC1 regulation via plasma membrane H+-ATPases in yeast and plant cells

Bruno André
Free University of Brussels

The TORC1 (Target of Rapamycin Complex 1) kinase complex plays a pivotal role in controlling cell growth in probably all eukaryotic species. The signals and mechanisms regulating TORC1 have been intensely studied in mammals, but those of fungi and plants are much less known. I will present recent data showing that the yeast plasma membrane H+-ATPase plays an important role in initial TORC1 activation in response to active nutrient uptake. Furthermore, I will provide evidence that homologous plant H+-ATPases, known to be regulated by practically all factors controlling cell growth, also contribute to TOR signaling.

Dynamics of transcription factors and the activation of the hematopoietic development

Petros Kolovos[1,*]
[1]*Department of Molecular Biology and Genetics, Democritus University of Thrace, Alexandroupolis, Greece*
**e-mail: pkolovos@mbg.duth.gr*

Transcription factors are important to control developmental processes. Here we focus on the role of various transcription factors in early hematopoietic development. These transcription factors form complexes which are present throughout life and during the entire process of hematopoietic development and differentiation. We performed functional studies at distinct hematopoietic stages and integrated multi-omics approaches to understand the regulatory roles of these transcription factors in developmental hematopoiesis. Our study provides new insights into how the combinatorial transcriptional regulatory network control a complex celldevelopmental process and drive the developmental lineage choice.

Role of ERAP1 in the autoimmune pathogenesis of Psoriasis

Univ.-Prof. Dr. Jörg Prinz
Department of Dermatology, University Clinics, Ludwig Maximilian-University of Munich, Frauenlobstr. 9-11, D-80337 Munich, Germany

Almost every autoimmune disease is associated with certain HLA alleles. Three of the more than 19,000 different HLA class I alleles confer a particular risk for immune-mediated diseases. HLA-B*27 is associated with ankylosing spondylitis, HLA-B*51 with Behcet's disease, and HLA-C*06:02 with psoriasis vulgaris. Gene-gene interaction between the respective HLA class I allele and variants of endoplasmic reticulum aminopeptidase 1 *(ERAP1)* further influence disease risk. Epistasis means that two independently inherited genes combine to change the phenotype or risk of a disease epistasis. The proteins encoded by the two genes have central functions in the antigen processing and presentation pathway. HLA class I molecules primarily present peptides from cytoplasmic proteins. Thus, a $CD8^+$ T cell response is primarily directed against target cells that can express these proteins and process them into antigenic peptides. NH_2-terminal trimming by ERAP1 may shorten precursor peptides to the length required for HLA presentation. The functional action and interaction of HLA-class I alleles and ERAP1 variants in autoimmune disease pathogenesis is unclear.
Psoriasis is a disease with inflammatory increased keratinocyte proliferation that can be completely revised by immunosuppressive therapy. It is mediated by clonal activation and expansion of $CD8^+$ T cells in the epidermis. Using a T-cell receptor from a lesional psoriatic $CD8^+$ T cell clone, we demonstrated that HLA-C*06:02 mediates a T_c17-type autoimmune response against melanocytes as the underlying pathomechanism of psoriasis through autoantigen presentation. The localization of melanocytes in the epidermis explains why psoriasis is primarily a skin-specific autoimmune disease. The melanocyte autoantigen presented by HLA-C*06:02 is a peptide from ADAMTS-like protein 5 which is selectively processed from the parental protein in melanocytes. In this process, we find that ERAP1 generates the melanocyte autoantigen by NH2-terminal trimming of precursor peptides to the length required for binding to the HLA-C*06:02 peptide binding groove. As a result, different ERAP1 haplotypes influence disease risk through different peptide yields due to different trimming activities. Furthermore, the expression of HLA-C proved to be much more dependent on ERAP1 activity, and thus on the ERAP1-generated fraction of the immunopeptidome, than overall HLA class I expression.
Taken together, the results from psoriasis explain the HLA class I association and its epistasis with ERAP1 of various immune-mediated inflammatory diseases through the presentation of certain ERAP1-dependent self-peptides, which can then be recognised by $CD8^+$ T cells. This makes ERAP1 a target molecule for the development of causal therapies.

Switching on and off DNA photo-degradation using quinazolin(on)e privileged pharmocophores. Towards novel photo chemotherapeutics and microbial photoinactivators

K. C. Fylaktakidou
Laboratory of Organic Chemistry, Chemistry Department, Aristotle University of Thessaloniki, 54124, Thessaloniki, Greece. E-mail: kfylakta@chem.auth.gr

Photosensitizers are compounds which absorb light of a specific wavelength. As a consequence, they become excited and subsequently they are able to trigger photo-physical phenomena or to initiate series of cascade reactions that find applications in solar cells, bio-imaging, chemical synthesis and biotechnology. In medicine, photosensitizers are used for photo-chemo and photo-dynamic therapies that constitute alternative and non or slightly invasive treatments for cancer and microbial infections. Crucially, light functions as an indispensable "on-demand" co-reactor as it acts in an accurate, spatial and temporal way. On the other hand, in drug design, photo-stability of drugs is considered important in order to avoid side effects in the organisms that receive the drug and are exposed to light in day activities. Thus, examining photo-sensitization abilities of chemical compounds remains either way of great importance.[1-3]
Quinazolin(on)es (QZ) are heterocyclic compounds found in numerous natural products and are categorized as "privileged" structures in medicinal chemistry due to their multiple and diverse biological activities.[4,5] QZ frame is considered as non-photo-responding[6] and thus studies on this direction are rather scarce.[7] Our team has an interest in photo-sensitization reactions which lately is focused on quinazolin(on)e derivatives.[8-12] Having examined various compounds with substituens on the aromatic frame as well as in the heterocyclic core and also having incorporated a variety of transition metals in certain positions we have evidence to support that several atoms, functional groups or metals may become buttons to switch on and off photo-sensitization reactions on quinazolin(on)es.
Our investigations include efficient synthetic methodologies and are supported by examination of the photo-chemical stability of the compounds, their affinity to DNA, photo-cleavage studies of plasmid DNA including mechanisms of action, normal and cancer cell viability in dark and upon irradiation and molecular docking theoretical calculations. These observations may lead to novel photo chemotherapeutics and microbial photoinactivators bearing the biocompatible quinazolin(on)e frame.

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The Complexity of Human Ribosomal RNA Biogenesis

Vassiliki Stamatopoulou
University of Patras

Ribosomal RNA (rRNA) biogenesis is absolutely essential for gene expression and disruption of this process irreparably leads to a new class of human diseases, termed ribosomopathies. An accurate pre-rRNA processing depends on a properly assembled nucleolus which requires the concerted action of essential enzymes, associated factors and epigenetic marks. Apart from the place where rRNA is synthesized and the initial steps of ribosome assembly take place, the nucleolus contributes to cell cycle regulation, modification of small RNAs, modulation of the telomerase function, nuclear export, tumor suppressor and oncogene activities. Due to its multilevel functionality and dynamic structure the nucleolus is considered a potent biomarker of cell transformation and a unique stress sensor. Therefore, we developed the "iNo score" tool to automatically describe the nucleolar morphology both quantitatively and qualitatively and identify trans-acting factors involved in the maintenance of the nucleolus structure. Our high-throughput screening revealed that depletion of around 40% of the human nucleolar proteins causes a severe nucleolar structure perturbation. Moreover, our software was be successfully used to identify novel factors and predict their role in the pre-rRNA synthesis.
Additionally, to better understand ribosome synthesis in human, we screened 680 putative nucleolar proteins for their involvement in pre-rRNA processing by Northern-blot analysis. This study identified 286 novel ribosome assembly factors, including 74 without yeast counterparts. Moreover, 38% of the identified factors have been connected to cancer and genetic disorders, while nearly 35% was found to perform additional or even entirely different functions than their yeast homologs. Strikingly, upon depletion of several late pre-40S assembly factors, such as the RNA helicase DHX37 and the splicing factor TSR1, we detected accumulation of aberrant rRNA fragments (rRFs) which call for further investigation. It is now known that the coordination and regulation of multiple biological processes depends on not only protein factors, but also on numerous regulatory non-coding RNAs. However, rRFs role in human cell homeostasis remains largely unknown. Therefore, our future plan is to clarify the function of these rRF molecules and highlight their putative correlation in human diseases and use as novel druggable targets.

Viruses and viral-like particles as delivery vehicles of RNAi in insects

Dimitrios Kontogiannatos, Anna Kolliopoulou, Luc Swevers
Insect Molecular Genetics and Biotechnology, Institute of Biosciences and Applications, National Centre for Scientific Research "Demokritos", Athens, Greece

RNA interference (RNAi) has now matured as a safe strategy for insect pest management that has several commercial products close to market release. Current strategies for insect pest control by RNAi are based on transgenic crops, also known as host-induced gene silencing. In parallel, non-transformative approaches of RNAi (spray-induced gene silencing) are also gaining momentum following continuously declining costs of dsRNA production. Nevertheless, a huge barrier for the application of dsRNA as an insecticide remains the efficient uptake by the targeted pests. To become economically viable for the majority of insect pests, new technologies need to be developed that increase the efficiency and potency of dsRNA in spray applications. In this presentation, efforts are described of the use of recombinant viruses (baculoviruses and RNA viruses) for the delivery of dsRNA molecules in insects. However, the application of recombinant viruses is controversial since they represent genetically modified organisms (GMOs) that receive high resistance from the public opinion in the European market. An attractive alternative is the employment of viral-like particles (VLPs) that encapsulate genetically inert dsRNA molecules as potentially highly efficient delivery vehicles. Efforts are described to produce VLPs based on the insect-specific Cypovirus and to synthesize long dsRNA cargo using the baculovirus expression vector system.

This work was supported 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-costresearch equipment grant" ("VLP-RNAi"; Project Number: 785).

Engineering cells, pathways and enzymes for the production of high-value compounds

Sotirios Kampranis
University of Copenhagen

Work in my lab aspires to develop Synthetic Biology as the method of choice for the synthesis of complex chemicals, replacing current organic chemistry methods that are inefficient and detrimental to the environment. To this end, we apply a multi-disciplinary approach that begins with the identification of biosynthetic enzymes, continues with the characterization and the engineering of the activities involved, and concludes with the reconstruction of biosynthetic pathways in biological systems for the sustainable synthesis of chemical compounds. In this lecture, I will present recent progress from my group in the engineering of yeast cells for the production high-value isoprenoids that have applications as pharmaceuticals, fragrances, flavours, or food and beverage ingredients.

The bone marrow stromal cell niche in Acute Myeloid Leukemia transformation, prevention and treatment

Stavroula Kousteni
Columbia University

Tumor cells can exploit their niches by remodeling their stromal components to activate pathways that favor cancer growth. Molecular delineation and targeting of those pathways may help overcome resistance to targeted therapies. In particular, acute myeloid leukemia (AML) -one of the most common leukemias in adults- remains recalcitrant to therapy, clinical management has barely changed for the last half century, and survival rates remain low. These shortcomings highlight the urgency of proposing novel therapies. Using genetic mouse models, patient-derived xenografts (PDX) and patient samples, we have identified a targetable leukemia-osteoblast crosstalk. Kynurenine, a tryptophan metabolite, is secreted by the leukemia cells which hijack the niche to their advantage, instructing the secretion by osteoblasts of the acute-phase protein serum amyloid A (SAA1). In turn, SAA1 activates a positive feedback loop on the AML cells perpetuating leukemia progression. Both Kynurenine and SAA1 fulfill the criteria of oncometabolites as their levels in the serum and bone marrow plasma increase in leukemia mouse models and patient-derived xenografts and correlate with disease progression from myelodysplastic syndrome (MDS) to AML patients. Moreover, pharmacological or genetic inhibition of the pathway in vivo hinders AML progression in PDX models. These results suggest that this niche-dependent, AML cell non-autonomous axis, can be exploited for the management of myeloid malignancies, opening new avenues for cancer treatment.

The bone marrow stromal cell niche in Acute Myeloid Leukemia transformation, prevention and treatment

Anastasios (Tassos) Pavlopoulos
IMBB-FORTH

During morphogenesis of multicellular organisms, cells integrate genetic and mechanical cues to produce the characteristic size and shape of developing tissues and organs. Our Developmental Morphogenesis laboratory aims to dissect the relative contribution and interplay of these physico-chemical mechanisms that orchestrate the emergence of biological form through patterned cell activities. We have introduced two genetically and optically tractable arthropod species, the shrimp-like crustacean Parhyale hawaiensis and the beetle Tribolium castaneum, as powerful and attractive model systems to study the molecular, cellular and mechanical basis of tissue and organ morphogenesis during animal development and evolution. We develop and integrate functional genetic and genomic tools with advanced light-sheet microscopy and image analysis pipelines to quantify developmental processes from a bottom-up cellular perspective, both in wild-type and in genetically or mechanically perturbed conditions. Our comparisons between Parhyale, Tribolium and other classic model systems in developmental biology have started shedding light on some common principles (or even deep homologies) in the underlying morphogenetic mechanisms by which animal tissues take shape during development, as well as on the evolution of these mechanisms to produce the stunning morphological diversity observed in nature.

Consequences of Telomere Shortening in Cancer and Aging in zebrafish

Miguel Godinho Ferreira
Institute for Research on Cancer and Aging Nice

Why, and how, organisms age and ultimately die is one of the key questions of modern biology. Telomeres are considered molecular timekeepers determining cellular lifespans. Disparate studies showed that telomeres shorten with age, DNA damage markers accumulate at telomeres and that animals lacking telomerase display compromised tissue function. An integrated organism-based detailed analysis is so far lacking. Here we show, for the first time, that short telomeres of specific tissues in naturally aged zebrafish precede DNA damage markers, decline in cell proliferation and age-specific organ decline. We used the telomere length of telomerase mutants to predict the critical threshold at which tissue dysfunction should arise in old individuals. Critically short telomeres accumulate specifically in the gut and muscle with aging, leading to cellular damage that culminates in local disruption of organ homeostasis. Importantly, telomere shortening in these key tissues appears to be sufficient to trigger damage in others and precedes the onset of age-associated diseases, namely cancer. Thus, tissue-specific telomere length is limiting for local and systemic physiological integrity, leading to tissue degeneration and disease in aging.

Regulation of fatty acids oxidation by nitric oxide. From bench to bedside

Paschalis-Thomas Doulias, PhD
[1]*Laboratory of Biochemistry, Department of Chemistry,*
[2]*Institute of Biosciences, University Research Center of Ioannina (U.R.C.I.), University of Ioannina, Ioannina 45110, Greece*

Nitric oxide (NO) is an endogenously formed gas that acts as a signaling molecule in the human body. The signaling functions of NO are accomplished through two primer mechanisms, the cGMP-mediated phosphorylation and the formation of S-nitrosocysteine on proteins.
Using innovative chemo-selective enrichment coupled with mass spectrometry we identified 1011 S-nitrosylation sites on 747 proteins in several organs of wild type mice. Functional enrichment analysis revealed a network of proteins participating to lipids metabolism and mitochondrial fatty acid oxidation (FAO). Interestingly, proteins participating to FAO were not S-nitrosylated in the corresponding tissues in mice lacking the endothelial nitric oxide synthase (eNOS-/-). eNOS-/- mice are hypertensive, hyperlipidemic and display insulin resistance. Moreover, they have reduced capacity to oxidize long chain fatty acids in the heart, liver and skeletal muscle. The functional consequences of S-nitrosylation were investigated using very long chain acyl-CoA (VLCAD) as a model protein. VLCAD catalyzes the first step of the fatty acids oxidation pathway. S-nitrosylation of VLCAD occurs on a single cysteine residue, is reversible and increases the catalytic efficiency of the enzyme by nearly 30-fold. Mechanistic insights are placed into a clinical context testing the efficacy of bioactive NO to augment enzymatic activity in the setting of VLCAD deficiency. VLCAD deficiency belongs to a collection of rare pediatric metabolic disorders known as FAO disorders. The data document the correction of enzymatic deficiency, the normalization of FAO capacity and the restoration of metabolic activity in VLCAD-deficient cells exposed to bioactive NO.
Overall, our data document that NO signaling regulates fatty acids metabolism. The pharmacological administration of bioactive NO to augment FAO and restore metabolic deficiency represents a promising area of investigation.

Neurovascular Interactions: Mechanisms, Imaging, Therapeutics

Katerina Akassoglou, PhD
Gladstone Institutes, University of California San Francisco

The communication between the brain, immune and vascular systems is a key contributor to the onset and progression of neurological diseases. We identified the coagulation factor fibrinogen as a blood-derived driver for neuroinflammation in a wide range of neurologic diseases, such as multiple sclerosis, Alzheimer's disease and brain trauma. We showed that fibrinogen is necessary and sufficient for neurodegeneration and a new culprit for microglia-mediated oxidative stress-dependent spine elimination and cognitive impairment. By developing Tox-Seq, we reported the oxidative stress innate immune cell atlas in neuroinflammation. We developed cutting-edge imaging tools to study brain network synchronization and the neurovascular interface. We discovered a first-in-class fibrin-targeting immunotherapy to selectively target inflammatory functions of fibrin without interference with clotting with efficacy in autoimmune- and amyloid-driven neurotoxicity. These findings could be a common thread for the understanding of the etiology, progression, and development of new treatments for neurologic diseases with neuroimmune and cerebrovascular dysfunction[1].

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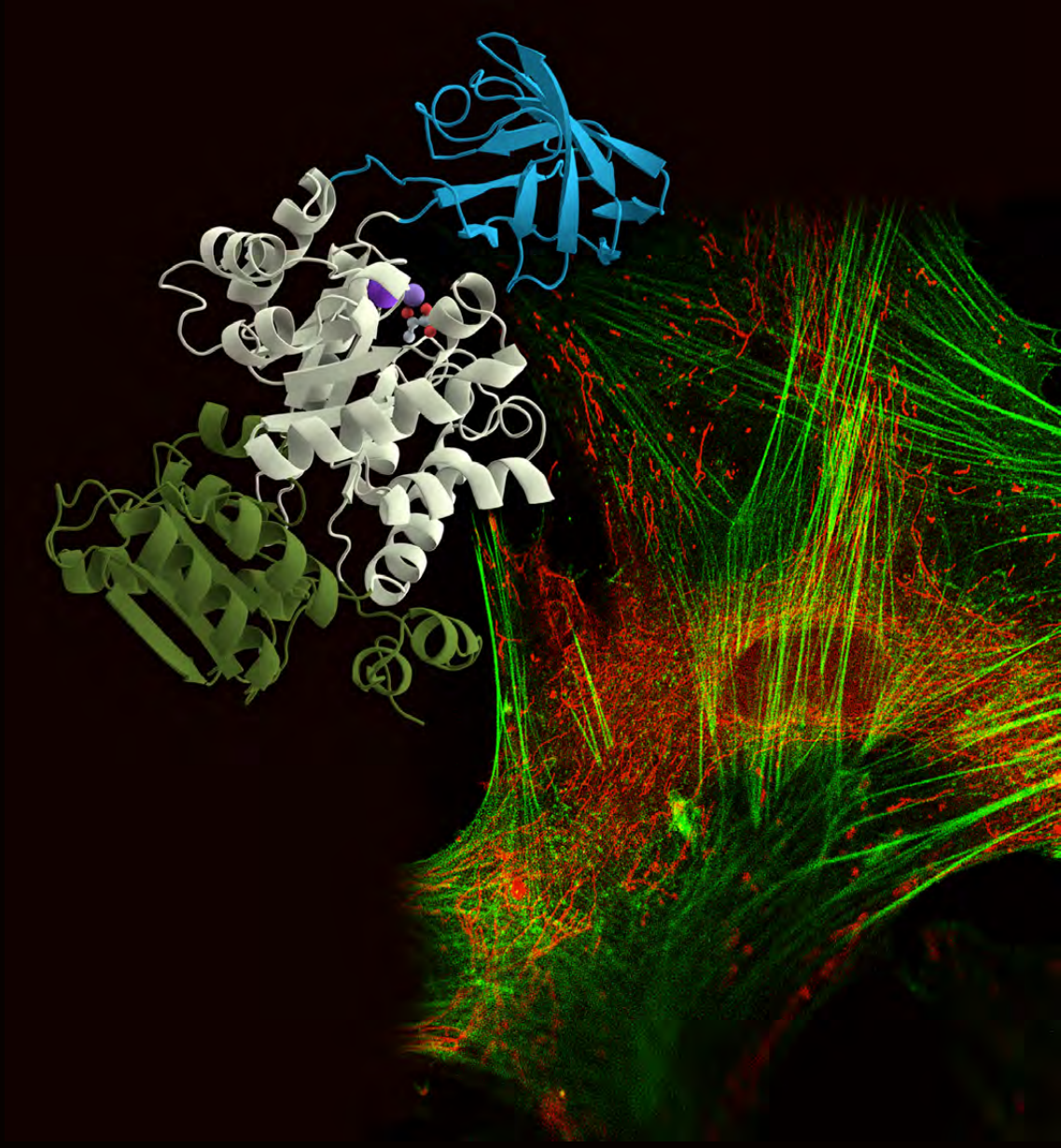

short talks_

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SHORT TALKS 1

Molecular & Cellular Basis of Human Diseases I

ST1

*Ld*PIBPsnx: The First Sorting Nexin in *Leishmania*

Olympia Tziouvara[1, 2*], Marina V. Petsana[3], Drosos Kourounis[1], Amalia Papadaki[1], Efthymia Basdra[2], Georgia G. Braliou[3], Haralabia Boleti[1, 4]

[1.] *Intracellular Parasitism Laboratory, Department of Microbiology, Hellenic Pasteur Institute, Athens, Greece*

[2.] *Department of Biological Chemistry, Medical School, National and Kapodistrian University of Athens, 11527 Athens, Greece*

[3.] *Department of Computer Science and Biomedical Informatics, University of Thessaly, 35131 Lamia, Greece*

[4.] *Light Microscopy unit, Hellenic Pasteur Institute, Athens, Greece*

Sorting nexins (SNX) are a large group of eukaryotic proteins implicated in several aspects of intracellular membrane trafficking and protein sorting. They all share a common phox homology (**ph**agocytic **ox**idase; PX) domain that binds to specific phosphoinositide (PI) lipids localized at different membrane surfaces of intracellular organelles. Many SNX family members also contain various other conserved structural domains, with BAR and FERM being the most prevalent ones. Although SNXs are evolutionary conserved they have been poorly studied in Protista.
We study the first SNX protein identified in *Leishmania* protozoan parasites, initially described as a PI-binding protein discovered in a **proteomic analysis of the secretome of *Leishmania (L.) donovani* (causative agent of** the fatal disease visceral Leishmaniasis). It is encoded by the *Ld*BPK_352470.1 gene. *In silico* prediction of its secondary and tertiary structures revealed that it **contains the PX domain and a BAR domain, structural features classifying it in the SNX-BAR subfamily of SNXs.** We named the *Ld*BPK_352470.1 gene ***Ld*PIBPsnx (*L. donovani*, PI Binding Protein sorting nexin)**.
Herein we present results confirming the expression and secretion of ***Ld*PIBPsnx by *L. donovani* promastigotes under different temperature and pH culture conditions mimicking the arthropod and mammal hosts' microenvironments.** The fact that ***Ld*PIBPsnx is secreted, highlights its value as a putative virulence factor. By immunofluorescence confocal microscopy using a specific anti-*Ld*PIBPsnx Ab that we generated we describe the intracellular** localization of the endogenous ***Ld*PIBPsnx** in ***L. donovani*** promastigotes and in macrophages infected with the parasites. Additionally, we examined the localization of heterologously expressed GFP-***Ld*PIBPsnx** in a transfected human cell line. All observed localizations suggest possible functions agreeing with the postulated SNX identity of ***Ld*PIBPsnx.** Sequence, structure and Evolutionary analysis revealed homology of *Ld*PIBP**snx and the human SNX2, while investigation of Protein-Protein Interactions utilizing STRING (v.11.5) predicted its putative molecular partners along with their functions in *Leishmania*.**

This work was funded by the Hellenic Foundation for Research and Innovation (HFRI) under the HFRI PhD Fellowship grant (Fellowship Number: 606) and "GENOMIC-OASIS: GENOMIC analysis of Organisms of Agricultural and liveStock Interest in Sterea", Grant Number (MIS) 5045902.

ST2

A-synuclein oligomers promote neuroinflammation by triggering the p38 MAPK pathway *in vivo*

Ioanna Chalatsa[1,2*], **Emmanouela Leandrou**[1,2], **Evangelia Emmanouilidou**[1,2]

[1] *Laboratory of Biochemistry, Department of Chemistry, National and Kapodistrian University of Athens, Panepistimiopolis* Zografou, 157 84, *Athens, Greece*

[2] *Neurodegenerative Diseases Division, Center for Basic Research, Foundation for Biomedical Research of the Academy of Athens, 4 Soranou Ephessiou Street, 115 27, Athens, Greece*

[*]*email: ichalatsa@biol.uoa.gr*

Parkinson's disease (PD) is the second major neurodegenerative disease. Although the mechanisms underlying PD are not fully understood, considerable evidence suggests that neuroinflammation is involved in the disease. In this study, we demonstrate for the first time that the endogenous α-synuclein oligomers present in A53T transgenic (Tg) mice striatum can trigger specific inflammation-related signaling pathways. Initial experiments indicated significant alterations in the number and morphology of microglia and astrocytes in the striatum of Tg mice compared with their wild type littermates. Western blot analysis in homogenized striatum of these mice after mild extraction with the zwitterionic detergent CHAPS showed the presence of SDS-soluble α-synuclein oligomers that was correlated with increased levels of mouse immunoglobulins indicative of an active immune response. RNA sequencing and subsequent bioinformatic analysis revealed the activation of a MAPK signaling pathway and a robust overexpression of PACAP neuropeptide and myocin light chain kinase (MYLK) the Tg tissue. RNA sequencing data were further confirmed by qPCR analysis. To investigate which signaling pathway could be potentially promoted by a-synuclein oligomers, we assessed the activation of the JAK/STAT3, CREB, STAT3 and p38 MAPK pathways by immunoblotting. Our results clearly indicated a profound activation of the p38 MAPK pathway in Tg striatum, whereas all other pathways remained unaltered. Key elements of this pathway, MSK1, ATF2/7 and p38 kinase were activated. Interestingly, the p65-NF-κB pathway was found to be significantly upregulated, whereas the A20/TNFAIP3 deubiquitinase, a critical negative regulator of NF-κB and inflammation was also underexpressed in Tg animals. Collectively, our data demonstrate that **α**-synuclein oligomers trigger signaling pathways of neuroinflammation in A53T Tg brain. Since inflammation is a basic component of the PD brain, elucidation of these complex pathways could provide novel therapeutic interventions to hinder disease progression.

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ST3

High Content Screening and Proteomic Analysis Identify a Kinase Inhibitor that rescues pathological phenotypes in a Patient-Derived Model of Parkinson's Disease

Nasia Antoniou[1], Kanella Prodromidou[1], Georgia Kouroupi[1], Ioanna Boumpoureka[1], Martina Samiotaki[2], George Panayotou[2], Maria Xilouri[3], Ismini Kloukina[3], Leonidas Stefanis[3,4] Regis Grailhe[5], Era Taoufik[1] and Rebecca Matsas[1]

[1]*Laboratory of Cellular and Molecular Neurobiology-Stem Cells, Hellenic Pasteur Institute, 127 Vassilissis Sofias Avenue, 11521 Athens, Greece*

[2]*Institute of Bioinnovation, Biomedical Sciences Research Center "Alexander Fleming", Vari 16672, Greece*

[3]*Center of Clinical Research, Experimental Surgery and Translational Research, Biomedical Research Foundation of the Academy of Athens (BRFAA), 4 Soranou Efesiou Street, 11527 Athens, Greece*

[4]*1st Department of Neurology, Eginition Hospital, Medical School, National and Kapodistrian University of Athens, Athens, Greece*

[5]*Technology Development Platform, Screening Sciences & Novel Assay Technology, Institut Pasteur Korea, Bundang-gu, Seongnam-si, Gyeonggi-do, 463-400 Republic of Korea*

Combining high throughput screening approaches with induced pluripotent stem cell (iPSC)-based disease modeling represents a promising unbiased strategy to identify therapies for neurodegenerative disorders. We have previously established a model of iPSC-derived neurons from patients with familial PD harboring the p.A53T αSyn mutation (G209A in the SNCA gene) that displays disease-relevant phenotypes at basal conditions. These included protein aggregation, compromised neuritic outgrowth, and contorted or fragmented axons with swollen varicosities containing αSyn and Tau (1). In this study we successfully adapted the p.A53T-iPSC-based cellular system in 384-well plate format and launched a screening campaign on a small kinase inhibitor library using high-content imaging. We thus identified the multi-kinase inhibitor BX795 that at a single dose effectively restores disease-associated neurodegenerative phenotypes. Proteomics profiling mapped the molecular pathways underlying the protective effects of BX795, comprising a cohort of 118 protein-mediators of the core biological processes of RNA metabolism, protein synthesis, modification and clearance, and stress response, all linked to the mTORC1 signaling hub. In agreement, expression of human p.A53T-αSyn in neuronal cells affected key components of the mTORC1 pathway resulting in aberrant protein synthesis that was restored in the presence of BX795 with concurrent facilitation of autophagy. Taken together, we have identified a promising small molecule with neuroprotective actions as candidate therapeutic for PD and other protein conformational disorders.

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ST4

A combined opposite targeting of p110δ PI3K and RhoA abrogates skin cancer

Niki Tzenaki[1], Lydia Xenou[1], George Valianatos[1], Evangelia Goulielmaki[1], Anna Tsapara[1], Maria Tzardi[2], and Evangelia A. Papakonstanti[1*]

[1]Department of Biochemistry, School of Medicine, University of Crete, Heraklion, Greece

[2]Department of Pathology, School of Medicine, University of Crete, University Hospital, Heraklion, Greece

Skin cancer includes melanoma and non-melanoma skin cancers. Malignant melanoma (MM) is the most aggressive and deadly skin cancer with an increasing incidence worldwide [1] and limited therapeutic options in advanced stage or metastatic patients [2]. Squamous cell carcinoma (SCC) belongs to non-melanoma skin cancers and it is caused by the cumulative life time exposure to ultraviolet radiation of the sun [3]. The prevalent accumulation of tumour-associated macrophages (TAMs) in MM has been confirmed to represent a poor indicator of patients' outcome whereas in non-melanoma cancers TAMs have also been considered to significantly contribute to disease development [4]. A wealth of evidence therefore suggests that combination approaches targeting both cancer cells and TAMs may be clinically beneficial [4-7]. Here we show that the development and metastasis of melanoma and SCC cancers can be blocked by a combined opposite targeting of RhoA and p110d PI3K. We find that a targeted induction of RhoA activity into tumours by deletion of p190RhoGAP -a potent inhibitor of RhoA GTPase [8]- in tumour cells together with adoptive macrophages transfer from $d^{D910A/D910A}$ mice in mice bearing tumours with active RhoA abrogated the growth and metastasis of melanoma and SCC tumours. These effects of the combined opposite targeting of RhoA and p110d are associated with suppressed proliferation and survival of tumour cells and blockade of the recruitment of macrophages to tumour sites. Together, our findings point to new ways of targeting cancer cells and macrophages for skin cancer therapy and illustrate the importance of p110d PI3K as a combinatorial regimen for the treatment of skin cancers.

This work is supported 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: 3405)

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ST5

The genomics β-pancreatic-T1D-hyper-Atlas of Virus-infection; a molecular-digital encyclopedia of T1D-related gene expression regulation

Marianna Koutsi[1], Spyros Foutadakis[1], Marialena Pouliou[1], Lydia Champezou[1], Ioanna Anastasiou[2], Dimitris Chatzopoulos[1], Apostolis Mourtzinos[1], Ioanna Eleftheriadou[2], Nikolaos Tentolouris[2], Ioannis Serafimidis[1], Marios Agelopoulos[1*]

[1] Biomedical Research Foundation Academy of Athens (BRFAA), 4 Soranou Ephessiou St., 115 27 Athens, Greece

[2] 1st Department of Propaedeutic Internal Medicine, Medical School, National and Kapodistrian University of Athens, Laiko General Hospital, Athens, Greece

** Corresponding author; email: magelo@bioacademy.gr*

Juvenile or Type 1 Diabetes (T1D) is a complex autoimmune disease caused by the destruction of β pancreatic cells and leads to a lifelong dependence of patients on exogenous insulin administration, without a causal link to a molecular mechanism yet being clearly shown. A trend in T1D research is based on the premise that environmental/extracellular stimuli such as pathogens, nutrition factors, pro-inflammatory molecules, etc. can predispose for the development of the disease by synergizing with the genetic background of individuals. Viruses are efficient agonists for the activation of antiviral, immune and inflammatory genes, which shape defense cellular responses that when altered, can lead to autoimmunity, and thus the hypothesis of their association with T1D development sounds reasonable (e.g. enteroviruses). Accordingly, our project aims to describe in-depth the early phases of the antiviral response of β pancreatic cells at the gene expression and epigenome levels. Our multidisciplinary research program is based on genomics, bioinformatics, and computational biology methodologies [(RNA-seq), (DNaseI-seq), (ChIP-seq), (Galaxy platform, R- and Linux-based reproducible workflows/pipelines, ROSE package and algorithms for sequencing data analysis)], as well as on in vivo functional massive-in-parallel validation approaches (STARR-seq) complemented by conservational comparisons and alignment to GWAS studies. Our results show that antiviral-, immune-response-, and β-pancreatic-specific genes are enriched among the pool of Differentially Expressed Genes (DEGs) identified upon Virus-infection in β pancreatic cells, and follow characteristic patterns of transcriptional induction, substantially instructed by their chromatin states. In addition, we managed to characterize de novo assembled and Virus-regulated enhancers and Super-enhancers (SEs) of the β pancreatic genome. Conclusively, our research succeeds in elegantly investigating the evolution of a gene expression program that accompanies Virus-infection of β pancreatic cells and in addressing epigenomic characteristics of chromatin landscapes, enhancers, and SEs assembly, and its completion is anticipated to assist the development of tailored diagnostic tools, drug design, and personalized therapeutic applications effective for T1D.

Keywords: Type 1 Diabetes, Viral Infections, β pancreatic cells, Chromatin and Gene Expression, Functional Genomics, Computational Biology, Bioinformatics

ST6

Unraveling the intricacy of the breast cancer transcriptome: novel circular RNAs of the *PRMT1* gene display a wide range of splicing events

Maria Papatsirou[1], Katerina Katsaraki[1], Christos K. Kontos[1], Dimitris Kletsas[2], Andreas Scorilas[1,*]

[1] *Department of Biochemistry and Molecular Biology, Faculty of Biology, National and Kapodistrian University of Athens, Athens, Greece*

[2] *Laboratory of Cell Proliferation & Ageing, Institute of Biosciences and Applications, National Centre for Scientific Research "Demokritos", Aghia Paraskevi, Greece*

Circular RNAs (circRNAs) constitute a type of RNA formed through back-splicing, a process in which the 5' donor splice site is joined to an upstream 3' acceptor splice site. Recent advances in high-throughput sequencing technologies and pipelines for data analysis have revealed that circRNAs are widely expressed in both normal and cancerous tissues. In breast cancer, circRNAs are proved to be implicated in tumor onset and progression. Although histone methylation by PRMT1 is largely involved in breast cancer development and metastasis, the effect of circular transcripts deriving from this gene has not been examined 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. For this purpose, total RNA was extracted from 4 breast cancer cell lines (BT-20, MCF-7, MDA-MD-468, and MDA-MB-231) and reversely transcribed with random hexamer primers. Next, first- and second-round PCRs were performed using gene-specific divergent primers, in order to selectively amplify *PRMT1* cDNAs resulting from circular transcripts. Sanger sequencing was then performed for the determination of the sequence of each novel *PRMT1* circRNA. Lastly, bioinformatical analysis was conducted to predict internal ribosome entry sites (IRES) and open reading frames (ORFs) in the novel circRNA sequences. In total, 9 novel circRNAs were identified, comprising both complete and truncated exons of the *PRMT1* gene. Interestingly, we demonstrated that all back-splice junctions exclusively consist of novel splice sites of the *PRMT1* exons, and mostly of non-canonical ones. Five out of these 9 *PRMT1* circRNAs were shown to possess a putative ORF. Moreover, the circRNA expression pattern differed dramatically among these 4 breast cancer cell lines. In conclusion, this study revealed the complete sequence of 9 novel circRNAs of the *PRMT1* gene, comprising distinct back-splice junctions and probably having different molecular properties.

SHORT TALKS 2

Systems Biology & Bioinformatics

ST7

CloudScreen; an "one stop shop" next generation platform for drug repurposing in the precision medicine era

Alexandros Kanterakis[1], Sofia Vasilakaki[2], Constantina Chalikiopoulou[3], Despoina Antonakaki[1,3], Sotiris Ouzounis[3], Jose Carlos Gomez Tamayo[4], Vivi Bafiti[3], Panagiotis Zoumpoulakis[3,5], Minos Timotheos Matsoukas[2], Theodora Katsila[3*]

[1]Institute of Computer Sciences, Foundation for Research and Technology Hellas, Heraklion, Greece
[2]Cloudpharm PC, Athens, Greece
[3]Institute of Chemical Biology, National Hellenic Research Foundation, Athens, Greece
[4]Research Programme on Biomedical Informatics (GRIB), Hospital del Mar Medical Research Institute (IMIM), Department of Experimental and Health Sciences, Universitat Pompeu Fabra, Barcelona, Spain
[5]Laboratory of Chemistry, Analysis & Design of Food Processes, Department of Food Science and Technology, University of West Attica, Greece
**email: thkatsila@eie.gr*

Drug repurposing is a state-of-the-art technology, capable of releasing the pharmaceutical industry from the current economic burden of developing new drugs and addressing adverse drug reactions. Notably, repurposed drugs may also reveal new molecular targets, expanding the pharmaceutical research and development portfolio. Drug repurposing consists of three phases: (a) the identification of a promising biomolecule for a given indication (working hypothesis phase), (b) the preclinical mechanistic evaluation of its bioactivity and (c) its efficacy evaluation in phase II clinical trials (if Phase I safety/ toxicity data exist in the context of the previous indication). Nowadays, a series of computational and experimental approaches are dedicated to drug repurposing, being pharmaco- or disease-centric. Current approaches are built on 1D data (genome-transcriptome-proteome-metabolome) and text mining (clinical trials) and may refer to all three aforementioned phases.
CloudScreen goes beyond existing strategies, as it:

(a) is not limited to 1D data, but curates/ integrates/ analyzes/ interprets 1D and 3D data coming from molecular design and computational chemistry outputs
(b) minimizes confounding in data mining/ integration/ analysis and promotes unbiased decision-making via the synergy of human and machine (artificial intelligence) reasoning capabilities (i) data mining services, ii) collaboration support services, and iii) decision making services
(c) provides dynamic update capabilities and extends text data services (patents, ethical and legal issues)
(d) serves as an "one-stop shop" tool for predicting and evaluating the efficacy and toxicity of repurposed biomolecules in new indications via medium-to-high throughput 3D-cell based ADME-Tox profiling

Our graph database platform consists of an architecture aiming to integrate such data and ultimately prioritizes drug repurposing candidates and new molecular targets by ranking predictive docking scores coupled to toxicity predictions and textual data. Our pipeline will be available as a web based one-stop-shop platform with a user-friendly GUI.

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ST8

Analysis for transcriptional alterations across bladder cancer stages identifies a pan-stage prognostic 8-gene panel and the gene AIF1 as candidate biomarker for immunotherapy selection

Rafael Stroggilos[1*], Maria Frantzi[2], Jerome Zoidakis[1], Marika Mokou[2], Emmanouil Mavrogeorgis[1], Anna Melidi[1], Manousos Makridakis[1], Maria G Roubelakis[1,3], Harald Mischak[2], Antonia Vlahou[1*]

[1.] *Biomedical Research Foundation of the Academy of Athens, Greece.*

[2.] *Mosaiques Diagnostics GmbH, Hannover D-30659, Hannover, Germany.*

[3.] *National and Kapodistrian University of Athens, School of Medicine, Greece*

Despite advancements in therapeutics, Bladder Cancer (BLCA) ranks as the most expensive cancer to treat, mainly due to multiple recurrences and to the lack of reliable biomarkers to guide patient management[1]. Precise panels of molecular biomarkers are in great need, in order to avoid unnecessary frequent visits or instead, to decide on aggressive therapeutic schemes from primary diagnosis. In search of putative prognostic markers, we investigated bladder carcinogenesis as a molecular continuum of transcriptional alterations forming through the disease stages. A total of 1,139 primary, well-characterized BLCA transcriptomes from 12 microarray studies were integrated and analyzed for monotonal alterations in gene expression, in pathway activities, and in coexpression patterns with increasing stage (Ta-T1-T2-T3-T4), starting from normal adjacent urothelium (NAU). Gene associations to outcome were investigated with Cox regression, and additional analysis for drivers of coexpression was performed. The TCGA-2017[2] and IMvigor-210[3] RNAseq data were used to validate findings.

We identified 157 genes and several pathways related prominently with cell cycle, showing a monotonically up- or down-regulated trend with higher disease stage. Eight monotonal genes (AKAP7, ANLN, CBX7, CDC14B, ENO1, GTPBP4, MED19, and ZFP2) associated with 5-year BLCA outcome both in the discovery and validation sets. Coexpression network analysis further detected intrinsic and microenvironmental gene rewiring programs operating variably across stages.These were linked with cell-cycle progression, extra-cellular matrix remodeling, regulation of metabolism and translation, as well as immune infiltration patterns. AIF1, a gene highly expressed during M2-polarization of macrophages[4], was identified as a driver of coexpression in the immune cells of T4 tumors, and its higher expression levels were associated significantly with complete response to immunotherapy.

Our findings indicate that decision for aggressive treatment may be dictated by a prognostic 8-gene panel, and additionally highlight AIF1 as a favorable marker in helping with patient selection for immunotherapy.

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ST9

A detailed phylogenetic and phylodynamic screening of the SARS-CoV-2 Alpha variant (20I) wave in Greece reveals interesting emerging sub-lineages with geographic tropism

Georgia Kontogianni*, Elena Gkotsi*, Bianca Passat*, Emmanouil Athanasiadis, Katerina Zoi, Giannis Vatsellas, Theodoros Loupis, Dimitris Thanos and Aristotelis Chatziioannou
Centre of Systems Biology, Biomedical Research Foundation of the Academy of Athens, Greece
**Equal contribution*

The emerging SARS-CoV-2 variants have increased transmissibility and virulence of the virus, affecting the performance of diagnostic tools and the effectiveness of vaccines. Initially a UK lineage, the Alpha variant has been acknowledged as a variant of concern carrying N501Y, P681H and numerous other mutations, spreading rapidly around the globe. Genomic surveillance offers the ability to detect and portray new lineages and variants of concern early on, allowing for effective implementation of control tactics. In this study, genomic data from the B.1.1.7 lineage in Greece were examined for genetic diversity by assessing gene mutations and inferring phylogeny, for the period from December 2020 to September 2021. Our analysis provides information about the epidemiological profile of SARS-CoV-2 in Greece, during the period when the Alpha variant was prevalent (beginning of February- till early Summer 2021), currently having receded to the presence of the Delta variant. The study exploited 12427 Greek patients' serum samples. Phylogenetic and phylodynamic analysis on the sequenced data was performed with iqtree and TreeTime, through augur. Most samples originated from Attica, impacting the structure of the phylogenetic tree, yet distinct clusters from other regions are formed, indicating an independent circulation of variants per division, contributing to the potential emergence of new sub-lineages, harbouring distinct combinations of mutations. Direct introduction of variants mainly from the USA and Switzerland is demonstrated, with the use of GISAID sequences, just as the exportation of novel variants originating from Greece, traced towards the USA and several European countries. Six mega-clades are identified, carrying disparate mutations mostly in the ORF1ab gene, but also critical events in the Spike and Nucleocapsid proteins with potential stabilising abilities and prospective key role in the host-cell interactions. Our findings also provide insights into the underlying dynamics of evolution of the Alpha variant, characterising important sub-lineages in Greece.

ST10

Viral genome assembly and characterization hindrances from virus-host hybrid reads; a refining approach

Nikolas Dovrolis, Katerina Kassela, Konstantinos Konstantinidis, Adamantia Kouvela, Stavroula Veletza, Ioannis Karakasiliotis
Laboratory of Biology, Department of Medicine, Democritus University of Thrace

Viral metagenomics, also known as virome studies, have yielded an unprecedented number of novel sequences, essential in recognizing and characterizing the etiological agent and the origin of emerging infectious diseases. Several tools and pipelines have been developed, to date, for the identification and assembly of viral genomes. Assembly pipelines often result in viral genomes contaminated with host genetic material, some of which are currently deposited into public databases. In the current report, we present a group of deposited sequences that encompass host RNA contamination. We highlight the detrimental role of chimeric next generation sequencing reads, between host RNA sequences and viral sequences, in virus genome assembly and we present the hindrances these reads may pose to current methodologies. We have further developed a refining pipeline, the Zero Waste Algorithm (ZWA) that assists in the assembly of low abundance viral genomes. ZWA performs context-depended trimming of chimeric reads, precisely removing their host moiety. These otherwise discarded reads were fed to the assembly pipeline and assisted in the construction of larger and cleaner contigs making a substantial impact on current assembly methodologies. ZWA pipeline may significantly enhance virus genome assembly from low abundance samples and virus metagenomics approaches in which a small number of reads determine genome quality and integrity.

Development & Differentiation

ST11

The interplay between Platelets, postnatal brain Neural Stem Cells of the Subependymal Zone and their niche

Christina Dimitriou[1,2]*, Maria Anesti[1], Maria Giachali[1], Cédric Ghevaert[3], Robin JM Franklin[2], Ilias Kazanis[1,2]

[1] *Laboratory of Developmental Biology, Division of Genetics Cell and Developmental Biology, Department of Biology, University of Patras, Patras, Greece*

[2] *Wellcome Trust – MRC Cambridge Stem Cell Institute & Department of Clinical Neurosciences, University of Cambridge, Cambridge, United Kingdom*

[3] *Wellcome Trust – MRC Cambridge Stem Cell Institute & Department of Haematology, University of Cambridge, Cambridge, United Kingdom*

**e-mail: chdimit12@gmail.com*

Postnatal brain Neural Stem Cells (pbNSCs) reside in specialized microenvironments, called stem cell niches, such as the Subependymal Zone (SEZ) of the lateral ventricles' walls. Previous work revealed specific aggregation of platelets (PLTs) within the niche's vasculature after focal demyelination in the adjacent corpus callosum (CC) and pro-survival effects exerted by PLT-derived factors on pbNSCs *in vitro*[1]. Here we report evidence of interaction between PLTs and pbNSCs, affecting the behaviour of the latter, using a co-culture system that allows us to assess the effects of their direct cell-to-cell interaction. Our analysis revealed that high PLT densities affect pbNSC proliferation and differentiation (both neurogenic and oligodendrogenic) potential, depending on the presence/absence of mitotic factors. When co-cultures were set up using Nbeal2$^{-/-}$-derived PLTs, characterized by non-functional α-granules, both effects were abolished. Moreover, experiments of CC demyelination in thrombocytopenic (Nbeal2$^{-/-}$, Crlf3$^{-/-}$) and thrombophilic (JAK2V6$^{fl/+}$) transgenic mice, followed by histological analysis of the SEZ and CC, showed reduced response of the SEZ vasculature in mice with altered numbers of circulating PLTs and deficient activation of oligodendrocyte progenitor cells (OPCs) in thrombocytopenic mice. We also assessed the presence of PLTs within the SEZ vasculature in other models of degeneration impacting the niche (post-stroke, after neuraminidase-induced ependyma disruption) or in the SEZ and in the non-typically neurogenic substantia nigra, of WT and of the parkinsonian "weaver" mice, especially after the administration of the proneurogenic microneurotrophin BNN-20. Finally, we observed activated PLTs inside blood vessels and in the brain parenchyma after their direct grafting in the SEZ and in the Striatum. In summary, we show that PLTs exert a functional role in the regulation of pbNSCs that is partially dependent on α-granules and their molecular compartments, and that is very likely mediated by, or is dependent on, the endothelium.

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ST12

Evaluation of plasma miR-146a and miR-155 as potential biomarkers for Mycosis Fungoides and detection of genetic variants (SNPs) in their genes

Chrysostomos Avgeros[1], Aikaterini Patsatsi[2], Kyriaki Ziampa[1], Dimitrios Dimitriadis[1], Triantafyllia Koletsa[3], Paraskevi Avgerou[1], Aikaterini Fragou[1], Theodoros Lialiaris[4], Georgios Tzimagiorgis[1], Elisavet Georgiou[1, 2]

[1]Laboratory of Biological Chemistry, School of Medicine, Aristotle University of Thessaloniki, Greece
[2]Cutaneous Lymphoma Clinic, 2nd Department of Dermatology and Venereology, "Papageorgiou" Hospital, School of Medicine, Aristotle University of Thessaloniki, Greece
[3]Department of Pathology, School of Medicine, Aristotle University of Thessaloniki, Greece
[4]Department of Genetics, School of Medicine, Democritus University of Thrace, Alexandroupolis, Greece

Introduction: Mycosis Fungoides (MF) constitutes the most common form of cutaneous T-cell lymphomas. The diagnosis is often challenging, making the use of molecular biomarkers essential for a timely diagnosis. The aim of the present study was to analyze plasma levels of miR-146a and -155 in MF patients and healthy volunteers and to detect the presence of SNPs in their genes.
Methods: The appropriate sample size was determined by a pilot study. The miRs' plasma expression was evaluated with qRT-PCR, using the $2^{-\Delta Ct}$ method and cel-mir-39 as reference gene. The promoter region and/or the pre-microRNA genomic region of these mirs were sequenced to detect the presence of SNPs. Statistical package SPSS 25 was used for analysis.
Results: Plasma levels of miR-146a and miR-155 were significantly higher in MF patients vs healthy controls, in early MF patients vs healthy controls and in advanced vs early MF patients (p=0,001 and p=0,028, p=0,001 and p<0,01, p=0,009 and p=0,002 respectively). Furthermore, plasma miR-146a and miR-155 considerably differentiated between MF stages (p=0,011 and p=0,047 respectively) and miR-155 was also remarkably altered between clinical skin manifestations. A positive correlation was detected between plasma levels of the two mirs in the patients' cohort (p<0.001). The AA genotype and the A allele in miR-155 rs767649(A>T) polymorphism as well as the GG genotype and the G allele in miR-146a rs2910164(C>G) polymorphism were significantly increased in MF patients and were associated with high risk of MF. Moreover, the genotypic combination (AA+GG) demonstrated higher distribution in MF patients and was correlated with an increased risk of MF.
Conclusion: The rs767649 and rs2910164 polymorphisms might be predisposing factors for MF. The detection of increased miR-146a and miR-155 plasma levels in MF patients is a promising finding in the attempt to establish noninvasive biomarkers for prompt diagnosis and prognosis of MF patients.

SHORT TALKS 3

Cell Communication & Signaling I

ST13

Xrp1, the transcription factor that links Ribosomal Defects to Cellular Responses and Cell Competition

Marianthi Kiparaki[1,2]*, Chaitali Khan[1], Virginia Folgado Marco[1], Jacky Chuen[1], Nicholas E. Baker[1]*
[1] *Albert Einstein College of Medicine, Bronx, NY, USA*
[2] *Institute of Cellular and Developmental Biology, Biomedical Sciences Research Center 'Alexander Fleming', Vari, Greece*

Cell competition is a universal phenomenon where cellular differences are sensed non-autonomously between neighboring cells in mosaic tissues and leads to active elimination of the less fit population. Cell competition is proposed to have both tumor-promoting and tumor-suppressive role, and also contribute in development, organ homeostasis, regeneration and aging. In 1975, cell competition was originally identified in mosaic fly tissues, where cells heterozygous for mutations in Ribosomal Protein (*Rp*$^{+/-}$) genes were eliminated by wild-type cells. We recently documented the remarkable finding that in *Rp*$^{+/-}$ cells, competition is not because of a direct response to reduced ribosome number, but rather a regulatory response controlled by the putative transcription factor Xrp1. Xrp1 activation is also responsible for the reduced translation and growth of Rp$^{+/-}$ cells and contributes to the developmental delay of Rp$^{+/-}$ flies. Recent work from Baker's lab established that cells with segmental aneuploidies in Drosophila are eliminated by the Xrp1-competition pathway on the basis of altered Rp gene dose. Here we show that Xrp1 reduced global translation in Rp$^{+/-}$ cells through PERK-dependent phosphorylation of eIF2α. eIF2α phosphorylation was sufficient to reduce translation in, and also enable cell competition in otherwise wild type cells. Unexpectedly however, we found that Xrp1 is also expressed in many other cellular defect conditions resulting in reduced ribosome biogenesis or function and eventually leads to increased eIF2α phosphorylation and elimination via cell competition. Thus, Xrp1, which is shown here to be a sequence-specific transcription factor, is the master regulator of ribosomal stress that triggers cell competition and other cellular responses, including the formation of protein aggregates observed in some ribosomal defects. These findings are not only important for elucidating cell competition mechanisms, but also have multiple implications for understanding and treatment of human ribosomopathies caused by mutations in components of the ribosome biogenesis pathway and function.

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ST14

Activation of the ATM-p53 axis is indispensable for the cytoprotection of UVB-exposed dermal fibroblasts

Eleni Mavrogonatou[1], Maria Angelopoulou[1], Harris Pratsinis[1], Sophia Rizou[2], Vassilis G. Gorgoulis[2], Dimitris Kletsas[1]*

[1] *Laboratory of Cell Proliferation and Ageing, Institute of Biosciences and Applications, National Centre for Scientific Research "Demokritos", Athens, Greece*

[2] *Molecular Carcinogenesis Group, Department of Histology and Embryology, Medical School, National and Kapodistrian University of Athens, Athens, Greece*

**e-mail: dkletsas@bio.demokritos.gr*

From the two solar ultraviolet (UV) fractions that can traverse the atmosphere and reach the earth's surface, the more energetic UVB photons are mainly absorbed by the skin epidermis, allowing only an approx. 5% to get to the upper layer of the dermis. Accordingly, the effect of UVB on the residing dermal fibroblasts' physiology has not yet been thoroughly investigated. Here we showed that exposure of human skin fibroblasts to UVB is cytotoxic *via* apoptosis, evidenced by DNA fragmentation and caspase-3 activation, which was also found to occur *in vivo*. UVB led to the phosphorylation of all members of the MAPK superfamily and of Akt, with Akt and JNKs being the key players in UVB cytoprotection. UVB-induced Akt and JNKs activation was validated *in vivo* in the skin of SKH1 hairless mice. Additionally, EGF receptor activation was proved to participate in the protective response of human dermal fibroblasts towards UVB, since its inhibition sensitized the cells, while exogenous supply of EGF rescued them from UVB treatment. UVB was shown to be genotoxic both *in vitro* and *in vivo* and the DNA damage response manifested by the activation of the ATM-p53 axis was linked with JNKs activation. Still, a functional DNA damage response was necessary for the long-term activation of Akt and JNKs and thus for the protection from UVB-mediated cell death. Accordingly, ATM inhibition or p53 loss-of-expression was detrimental for skin fibroblasts. The Akt activator SC79, which was found here for the first time to activate also JNKs, reversed to an extent UVB-induced cytotoxicity and the Nrf2 inducer sulforaphane provided an extra protection to skin fibroblasts. On the other hand, although UVB radiation increased intracellular ROS levels, classical antioxidants did not provide any protection to the cells. Our findings will hopefully aid the design of novel photoprotective approaches.

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ST15

IL-17 and IFNα-regulation signaling pathways' enrichment highlights a potent immunomodulatory role of exosomes in *Leishmania*-infected macrophages

Antonia Efstathiou[1], Dimitra K. Toubanaki[1], Martina Samiotaki[2], George Stamatakis[2], Evdokia Karagouni[1*]
[1]*Hellenic Pasteur Institute, Department of Microbiology, Vas. Sofias 127, 11521, Athens, Greece*
[2]*Institute of Bioinnovation, Biomedical Sciences Research Center "Alexander Fleming", Vari, 16672, Greece*
**e-mail: ekaragouni@pasteur.gr*

Exosomes are known to play a crucial role in cellular communications in eukaryotic cells, however their role is not yet fully elucidated. This type of communication can be exploited by microorganisms like *Leishmania* during the establishment of infection in the host. *Leishmania*, a eukaryotic parasite which is the causative agent of leishmaniasis, is introduced to the host through a sand-fly blood meal, depositing the infectious metacyclic promastigote form of the parasite in the skin which eventually enter a variety of host cells, mostly macrophages where the parasite replicates intracellularly during chronic leishmaniasis. Like other eukaryotes, *Leishmania* promastigotes releases exosomes, while infected-macrophage exosomes are believed to have leishmanial protein and miRNA cargo. Aim of this study was to investigate the protein cargo of exosomes derived from cell cultures of infected macrophages with *Leishmania infantum*, in order to evaluate its potential immunomodulatory role during the infection and its possibility to be exploited as vaccine candidate. Upon isolation, exosomes were extensively characterized and subsequently analyzed by LC-MS/MS. An enriched network of host proteins participating in key role signaling pathways was emerged during the data analysis. More specifically, six proteins belonging in the IL-17 signaling pathway including TNF receptor-associated factor 6 and Caspase-3, were highly enriched in the exosomes. Moreover, the upregulation of five positive and the downregulation of one negative IFNα-signaling pathway regulator, reveal a potent tendency of the exosomes to promote this pathway. Interestingly, both IL-17 and IFNα pathways can promote the establishment of a *Leishmania* infection, thus exosomes seem to play an active immunomodulatory role probably in favour of the parasites during the infection of the macrophages.

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ST16

Inhibition of hyaluronan biosynthesis inactivates ribosomal protein S6 in metastatic breast cancer cells

Athanasios Chatzopoulos[1,2#], Theodoros Karalis[2#], Paraskevi Heldin[2], Spyridon S. Skandalis[1*]
[1] Biochemistry, Biochemical Analysis & Matrix Pathobiology Res. Group, Laboratory of Biochemistry, Dept. of Chemistry, University of Patras, Greece
[2] Dept. of Medical Biochemistry and microbiology, Uppsala University, Sweden
[#] equal contribution
** E-mail: skandalis@upatras.gr*

Hyaluronan is a predominant component of the extracellular matrix (ECM). 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 part of the higher eukaryotic 40S ribosomal subunit which is phosphorylated in 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) and salicylate, which are inhibitors of hyaluronan biosynthesis. Our preliminary data showed that both inhibitors reduced the phosphorylation status of rpS6 followed by the substantial suppression of breast cancer cell proliferation and growth. Furthermore, the treatment with 4-MU and salicylate resulted in cell cycle arrest as evidenced by FACS analysis and the reduced protein levels of cyclin D1, which has central roles in the regulation of cell cycle progression. Notably, 4-MU and salicylate both decreased protein synthesis in MDA-MB-231 cells.
These findings indicate a critical regulatory role for hyaluronan in metastatic breast cancer cell growth.

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ST17

Comparative NMR study of SARS-CoV, MERS-CoV and SARS-CoV-2 macro domains with ADPr and a putative inhibitor

Periklis Charalampous, Aikaterini C. Tsika, Angelo Gallo, Nikolaos K. Fourkiotis, & Georgios A. Spyroulias*
Department of Pharmacy, University of Patras, 26504 Patras, Greece

Macro domains (MDs) are conserved structural motifs found in viruses as well as in all kingdoms of life. They counteract/revert the ADP-ribosylation, catalyzed by poly-ADP-ribose polymerases (PARPs), which is an antiviral mechanism of the host cell. According to crystallographic characterization of the MDs of SARS-CoV, MERS-CoV and SARS-CoV-2 (three members of the betacoronavirus genus) they possess similar overall fold, but they also exhibit interesting differences in their ADPr binding clefts (Alhammad et al. 2021). In this study we report the NMR backbone assignment (^{1}H, ^{13}C, ^{15}N) of the SARS-CoV, MERS-CoV and SARS-CoV-2 macro domains in the free and ADPr bound forms as well as their NMR chemical shift based secondary structure prediction. We have also performed chemical shift perturbation analysis of the macro domains from these three betacoronaviruses to monitor which residues are involved in the ADPr and in a putative inhibitor binding. The study of these features is important to identify unique patterns and motifs that will allow the determination of pharmacophore characteristics that can be used to design selective antiviral drugs against current or future coronavirus diseases.

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ST18

Breakthrough use of natural human monoclonal IgGs penetrating MDA-MB-231 cells as anti-neoplastic agents or as intracellular vehicles of gold nanoparticles loaded with paclitaxel

Theodora Stivarou[1], Ligeri Papaioannou[2#], Ioannis Sarrigeorgiou[1#], Konstantinos Avgoustakis[2, 3] and Peggy Lymberi[1*]

[1]*Immunology Laboratory, Department of Immunology, Hellenic Pasteur Institute, 11521 Athens, Greece*

[2]*Laboratory of Pharmaceutical Technology, Department of Pharmacy, University of Patras, 26504 Patras, Greece*

[3]*Research Foundation Academy of Athens (BRFAA), 4 Soranou Ephessiou Street, Athens 11527, Greece*

[*]Corresponding author: plymberi@pasteur.gr

[#]These authors equally contributed to this work

Our laboratory has been focused on the study of human natural, polyreactive monoclonal IgGs (mIgGs) regarding their cell-penetrating ability and effect on metastatic breast cancer cells migration and viability. Their use as intracellular transporters of gold nanorods (GNRs) carrying anticancer drugs, such as paclitaxel (PTX), was further studied. The conjugation of mIgGs on these nanoparticles would increase the intracellular delivery of the anticancer drug to cancer cells.

A series of mIgGs from Multiple Myeloma-G (MM-G) patients' sera were isolated by protein-G affinity-chromatography, checked for a) purity and monoclonality (by SDS-PAGE and IEF, respectively), b) polyreactivity against self- and non-self- antigens (by ELISA), and c) cell-penetrating capacity (by immunofluorescence (IF) experiments on live MDA-MB-231 metastatic breast-cancer cells) and d) effect on apoptosis and cell migration (by flow-cytometry and wound-healing assays, respectively). mIgGs with cell- penetrating ability (CPAbs) were coupled on -average length 28 nm- GNRs surface, and the CPAb-capacity and cytotoxicity of CPAbs-GNRs conjugates were visualized through IF-experiments on live MDA-MB-231 cells.

Among 41 mIgGs, 19 had cell-penetrating capacity with distinct cytoplasmic localization. Three CPAbs inhibited while other two enhanced MDA-MB-231 cell migration; these 3, also dramatically induced cell-death. CPAbs that had no effect on cell-motility or viability were successfully conjugated to GNRs. CPAbs-GNRs conjugates exhibited an enhanced cell-penetrating ability compared to the unconjugated CPAbs. Furthermore, CPAbs-GNRs loaded with PTX induced a significantly increased rate of mortality on the cancer cells with respect to the controls.

Overall, human natural mIgGs with cell-penetrating capacity could be exploited as advantageous, due to their natural origin, potential anti-neoplastic tools. CPAbs could be used either *per se*, or as vehicles for intracellular delivery of anticancer drugs, or both for a combined, effective cancer immunotherapy with less toxicity.

ST19

Mutational analysis reveals the substrate translocation trajectory and a stabilization role of a C-terminal transmembrane domain in a NCS1 nucleobase transporter

Yiannis Pyrris, Georgia Papadaki, George Diallinas
Department of Biology, National and Kapodistrian University of Athens, Panepistimioupolis 15784, Athens, Greece

FurE is a H^+ symporter specific for the cellular uptake of uric acid, allantoin or uracil, but also of nucleobase analogues of pharmacological importance (e.g. 5-fluorouracil), in the model microbial eukaryote ***Aspergillus nidulans***[1,3]. Being a member of the well-studied NCS1 family of transporters[1] (part of the APC-superfamily[4]) FurE is believed to function via the so-called ***rocking-bundle mechanism***[5]. Structural models of FurE in inward-, occluded and outward-facing conformations have been generated by homology modelling based on the crystal structures of the bacterial homolog Mhp1[6,7]. Previous genetic studies, substrate-docking methods and preliminary Molecular Dynamics, have led to the identification of residues involved in substrate binding, as well, as residues affecting transport dynamics, substrate specificity and the endocytic turnover of FurE[2,3]. The present study aims to provide insights into the structure of the substrate translocation trajectory, and investigate the role of last two, little-studied, transmembrane domains (TMS11 and TMS12) of FurE. Functional analysis of rationally designed mutations based on the modeling of FurE in various conformations, showed that the residues modified are significant for substrate specificity and/or transport. Our findings highlight how ***in silico*** structural approaches combined with tangible genetic evidence provide powerful tools for the clarification of structure-function relationships in transporters. We also performed systematic alanine-scanning mutagenesis in TMS11 and TMS12 and functional analysis of respective mutants, which showed that these segments are largely dispensable for transport activity. However, specific residues proximal to extracellular and cytoplasmic loops of TMS11 and TMS12, respectively, were found to be crucial for substrate specificity. Our analysis also identified a critical role of Y484 in FurE proper folding. Modeling and genetic evidence suggest that Y484 participates in polar and hydrophobic interactions essential for the structural integrity of the protein. The stabilizing role of TMS11-12 might well extend to other APC-superfamily transporters employing a rocking-bundle mechanism.

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ST20

Unraveling BCL2-ovarian killer (*BOK*) alternative splicing pattern in human cancer cell lines, using targeted nanopore sequencing

Pinelopi I. Artemaki, Andreas Scorilas, Christos K. Kontos*
Department of Biochemistry and Molecular Biology, Faculty of Biology, National and Kapodistrian University of Athens, Athens, Greece

Alternative splicing is a process responsible for the increase in the diversity of the coding and non-coding cellular landscape. Accurate alternative splicing profiles and regulation determine cellular fates and functions, while its deregulation is a cancer hallmark. The products of alternative splicing usually encode distinct protein isoforms with different – sometimes even opposite – functions. *MCL1* and *BCL2L1* (*BCLX*), members of the BCL2 family, constitute two prominent examples. BCL2 family proteins play a pivotal role in the intrinsic apoptotic pathway. One intriguing member of this family is BCL2-ovarian killer (BOK). BOK is a pro-apoptotic factor, yet an anti-apoptotic function has been described, as well. This ambiguity could be attributed to the presence of multiple BOK protein isoforms with different structures, encoded by alternatively spliced transcripts. However, most studies focus on protein function, neglecting RNA molecules. Prompted by this, we decided to investigate *BOK* splicing pattern in two of the most common malignancies, since its implication in cancer is quite interesting. For this purpose, 5μg of total RNA were isolated from 5 human colorectal cancer cell lines and 3 prostate cancer ones, and were reversely transcribed. Next, we performed nested PCR, targeting the coding sequence of *BOK*. Third-generation sequencing using nanopore technology was conducted, and the results were bioinformatically analyzed. This analysis revealed distinct splicing patterns between colorectal and prostate cancer cell lines, and even among cancer cell lines with common tissue of origin. Moreover, alternative 3'- and 5'-splice sites with high occurrence emerged. Interestingly, exon 2, which is supposed to bear the translation start codon (AUG), possesses several internal 3'-splice sites and an internal intron, which constitute typical features of a 5'-untranslated region. Overall, these findings raise questions regarding the main transcripts and the respective protein isoforms of human *BOK*, and pave the way for further investigation of their function and localization.

SHORT TALKS 4

Functional Genomics and Proteomics

ST21

Evaluating the effect of 17-AAG HSP90 inhibition in the abundance of heat shock proteins in Mantle Cell lymphoma model cells line using comparative proteomics

Sarantis Tsetsakos[1,2,3], George Stamatakis[4], Martina Samiotaki[4], Konstantina Psatha[1,2,5], Michalis Aivaliotis*[1,2,5]

[1]Laboratory of Biological Chemistry, School of Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece

[2]Functional Proteomics and Systems Biology (FunPAth), Center for Interdisciplinary Research and Innovation (CIRI-AUTH), Thessaloniki, Greece

[3]Department of Molecular Biology and Genetics, Health Sciences School, Democritus University of Thrace, Alexandroupolis, Greece

[4]Institute of Bioinnovation, Biomedical Sciences Research Center "Alexander Fleming", Athens, Greece

[5]Institute of Molecular Biology and Biotechnology, FORTH, Heraklion, Greece

[*]Correspondence: aivaliotis@auth.gr

Heat Shock Proteins (HSPs) is a family of molecular chaperones[1], essential to survival under stressful conditions and regulation of apoptosis[2]. Conversely, HSPs are also implicated in oncogenesis[3],[4]. They are reportedly over-expressed in several tumors, where they support apoptosis evasion and resistance to chemotherapeutics or radiation[5]. Proteins with an established role in tumor progression are demonstrated client-proteins of HSP90[6]. Therefore, HSP-inhibitors have emerged as a promising class of therapeutic agents[5],[7]. However, malignant cells often overcome targeted HSP-inhibition by increasing the expression of other HSPs.

Here, we report the effect of HSP90 inhibitor 17-AAG[8],[9] on the viability and proteostasis of human mantle cell lymphoma (MCL) cells expressing wild-type p53 (JMP-1 line). Specifically, we focus on the 17-AAG-induced effect on the expression of HSPs[10]. We treated cells with 1 and 2μM 17-AAG for 24-48 hours, and also with Nutlin-3a, a potent p53 activator[12], and assessed their viability. Subsequently, we extracted and quantified their DNA, RNA and protein content. After FASP and in-solution tryptic digestion, protein samples were analyzed by quantitative mass spectrometry-based proteomics, using nLC-ESI-MS/MS[11], and further protein identification and relative quantitation was implemented by advanced bioinformatics. Western Blotting (WB) was performed for results' verification.

We consistently observed a dose- and time-dependent decrease of viability and cell density after treatment with 1 and 2uM 17-AAG for 24h-48h. Combining 17-AAG and N3a demonstrated additive effect on the diminution of the aforementioned parameters. Our preliminary proteomic analysis identified more than 55 different HSPs and their isoforms and revealed their regulation after the treatment. Several HSPs, ***including HSP90,*** and HSPs-related proteins were downregulated; while others, ***including HSP70,*** were upregulated, following 17-AAG treatment. These finding were also confirmed by immunoblotting. However, discrepancies in the expression of HSPs across treatment groups were also observed, reflecting the development of ***complex*** protection strategies under stressful conditions. Such an approach exploits the potential role of (targeted) proteomics linking HSPs inhibition and tailored therapeutic interventions in MCL lymphoma.

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ST22

Novel components of cell-matrix adhesions revealed by proteomic analysis

G. Rouni[1], M. Samiotaki[1], V. Kostourou[1]*
[1] *BSRC Alexander Fleming, Vari, Greece*
**e-mail:kostourou@fleming.gr*

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 cystoskeletal 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 homostasis 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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ST23

An ERAP2 inhibitor induces cell-surface presentation of many new and potentially antigenic peptides by cancer cells

Ioannis Temponeras[1,2], George Stamatakis[3], Martina Samiotaki[3], Harris Pratsinis[1], George Panayotou[3] and Efstratios Stratikos[1,4,*]

[1] *National Centre for Scientific Research "Demokritos", Agia Paraskevi, Attica, Greece*
[2] *Department of Pharmacy, University of Patras, Patra, Greece*
[3] *Biomedical Sciences Research Center "Alexander Fleming", Vari, Attica, Greece*
[4] *Department of Chemistry, National and Kapodistrian University of Athens, Greece*
[*] *Corresponding Author: E-mail: estratikos@chem.uoa.gr or stratos@rrp.demokritos.gr.*

The cellular immunopeptidome, the sum of peptides presented on the cell-surface by Major Histocompatibility Class molecules, is central to adaptive immune responses. ER aminopeptidase 2 is an intracellular enzyme that regulates the cellular immunopeptidome by processing the N-terminus of antigenic peptide precursors and antigenic peptides. Recent studies have linked reduced ERAP2 expression levels to increased efficacy of immune-checkpoint inhibitor cancer immunotherapy, raising the exciting possibility that pharmacological inhibition of ERAP2 could enhance the efficacy of cancer immunotherapy and extend it to more patients. To explore the effects of chemical ERAP2 inhibition on the cellular immunopeptidome of cancer cells we treated the MOLT4 T lymphoblast leukaemia cell line with a recently developed selective ERAP2 inhibitor, DG011A, isolated MHC class I molecules and sequenced bound peptide by tandem liquid chromatography mass spectrometry. Inhibitor treatment only marginally affected MHC class I presence on the cell surface but induced significant shifts on the immunopeptidome so as >20% of detected peptides were either novel or significantly upregulated. Most of the inhibitor-induced peptides were 9mers and had predicted affinity and sequence motifs consistent with being optimal ligands for at least one of the MHCI alleles carried by MOLT4 cells. These inhibitor-induced MHCI-presented peptides may serve as triggers for novel cytotoxic responses against cancer cells and thus synergize with the therapeutic effect of immune-checkpoint inhibitors.

Ageing

ST24

Extra Virgin Olive Oil consumption from Mild Cognitive Impairment patients attenuates oxidative and nitrative stress reflecting on the reduction of the PARP and DNA damage levels

Elena E. Tzekaki[1,4], Magda Tsolaki[2,3,4], George D. Geromichalos[1], Anastasia A., Pantazaki[1,4*]

[1] *Laboratory of Biochemistry, Department of Chemistry, Thessaloniki, Greece*

[2] *1st Department of Neurology, "AHEPA" General Hospital Medical School, Aristotle University of Thessaloniki, Thessaloniki, Greece*

[3] *Greek Association of Alzheimer's Disease and Related Disorders – GAADRD, Thessaloniki, Greece*

[4] *Center for Interdisciplinary Research and Innovation, Laboratory of Neurodegenerative Diseases (LND), Thessaloniki, Greece*

Alzheimer's disease (AD) constitutes a neurodegenerative disorder with detrimental consequences resulted in loss of neurons. Oxidative/nitrative stress that stems from the unbalanced overproduction/clearance of reactive oxygen/nitrogen species (ROS/RNS) have a pivotal role in this multifactorial disorder. Therefore, DNA lesions produce DNA damages and disruption in cellular function, frequent in AD brain. 8-hydroxy-2´-deoxyguanosine (8-OHdG) is recommended as a marker of DNA lesions while the enzyme NOS2 (Nitric oxide synthase 2) of nitrative stress. In condition of massive oxidation, poly(ADP-ribose)polymerase 1 (PARP-1) is activated for further recruiting DNA repair enzymes. PARP-1 determines the cell's fate- survival or death- and it is positively correlated with neurodegeneration. In this clinical trial, we investigated the possibility to inhibit the disease on its onset through the administration of extra virgin olive oil (EVOO) in Mild Cognitive Impairment (MCI) patients. For this reason, we utilized a wide bank of MCI patients' sera who were administered EVOO to examine its potential effects. We found that PARP-1 levels decrease to the normal levels in MCI patient serum after EVOO treatment for 12 months, and similarly the levels of 8-OHdG followed the same tendency. Additionally, the nitrative stress may be suppressed as NOS2 concentration lessened in MCI group after EVOO administration. Importantly, AD-related biomarkers (Aβ1–42 and p-tau) are meliorated after administration of EVOO in MCI patients for 12 months. I*n silico* analysis has proved the binding of EVOO constituents on PARP-1 and NOS-2 enzymes and their interaction with crucial amino acids of the active sites. Conclusively, we provide clinical trial evidence confirming that annually EVOO intake by MCI patients restores DNA oxidative damage reducing 8-oxo-dG levels, and attenuates nitrative stress through iNOS reduction thanks to its multifunctional role (antioxidant, anti-inflammatory), and may be a potential therapeutic approach against neurodegeneration leading to AD.

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Elena E. Tzekaki, Magda Tsolaki, George D. Geromichalos, Anastasia A. Pantazaki(2021) Extra Virgin Olive Oil consumption from Mild Cognitive Impairment patients attenuates oxidative and nitrative stress reflecting on the reduction of the PARP levels and DNA damage. Experimental Gerontology (in press)*

ST25

Nrf2 activation induces mitophagy and reverses Parkin/Pink1 knock down-mediated neuronal and muscle degeneration phenotypes

Sentiljana Gumeni, Eleni-Dimitra Papanagnou, Maria S Manola, Ioannis P. Trougakos
Department of Cell Biology and Biophysics, Faculty of Biology, National and Kapodistrian University of Athens 15784, Greece

The balanced functionality of cellular proteostatic modules is central to both proteome stability and mitochondrial physiology; thus, the age-related decline of proteostasis also triggers mitochondrial dysfunction, which marks multiple degenerative disorders. Non- functional mitochondria are removed by mitophagy, including Parkin/Pink1-mediated mitophagy. A common feature of neuronal or muscle degenerative diseases, is the accumulation of damaged mitochondria due to disrupted mitophagy rates. Here, we exploit Drosophila as a model organism to investigate the functional role of Parkin/Pink1 in regulating mitophagy and proteostatic responses, as well as in suppressing degenerative phenotypes at the whole organism level. We found that Parkin or Pink1 knock down in young flies modulated proteostatic components in a tissue-dependent manner, increased cell oxidative load, and suppressed mitophagy in neuronal and muscle tissues, causing mitochondrial aggregation and neuromuscular degeneration. Concomitant to Parkin or Pink1 knock down cncC/Nrf2 overexpression, induced the proteostasis network, suppressed oxidative stress, restored mitochondrial function, and elevated mitophagy rates in flies' tissues; it also, largely rescued Parkin or Pink1 knock down- mediated neuromuscular degenerative phenotypes. Our in vivo findings highlight the critical role of the Parkin/Pink1 pathway in mitophagy, and support the therapeutic potency of Nrf2 (a druggable pathway) activation in age-related degenerative diseases.

SHORT TALKS 5

Cell Communication & Signaling II

ST26

LonP1 disruption triggers mitochondrial and cytoplasmic stress responses interfering with cell survival and motility in cancer cells

Stefanos Smyrniotis[1], Labrina Bondi[1,2], Socratis Avgeris[1], Eleni Kyriakou[2], Eirini Taouktsi[2,3], Fivos Borbolis[2], Popi Syntichaki[2], Gerassimos E. Voutsinas[1*]

[1]Laboratory of Molecular Carcinogenesis and Rare Disease Genetics, Institute of Biosciences and Applications, National Center for Scientific Research Demokritos, Aghia Paraskevi Attikis, Greece; [2]Biomedical Research Foundation of the Academy of Athens, Laboratory of Molecular Genetics of Aging, Center of Basic Research, Greece; [3]Agricultural University of Athens, Greece

**e-mail: mvoutsin@bio.demokritos.gr*

LonP1, an ATP-dependent protease of the mitochondrial matrix, which also possesses chaperone and mitochondrial DNA integrity maintenance activities, was demonstrated to be positively involved in protein homeostasis and upregulated under stress conditions. Since these mechanisms are vital for cancer initiation and progression, playing important roles in cell survival and function, we sought to investigate the status of stress response mechanisms in mitochondria and the cytoplasm after LonP1 inhibition with the use of the synthetic triterpenoid CDDO-Me (Bardoxolone Methyl) or genetic silencing of LonP1 with siRNA. We first examined cytotoxicity of CDDO-Me on HT1080 (fibrosarcoma) and WM266-4 (metastatic melanoma) cells using the MTT assay. Then, we analyzed expression of genes involved in cellular stress response processes with the help of RT-qPCR and western blots. We found significant increases of expression in genes involved in mitochondrial stress responses (UPR^{mt}), as well as induction of cytoplasmic stress responses (ISR), redox machinery, cytoplasmic heat shock proteins and autophagy. We also observed upregulation or activation of proteins controlling cellular responses involved in stress and apoptosis. Finally, we demonstrated that LonP1 inhibition by CDDO-Me interferes with motility of cancer cells. These results demonstrate that LonP1 disruption activates stress responses, but also interferes with cell survival and motility in cancer cells, whereas it also designates promising anticancer effects of CDDO-Me.

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ST27

RASSF1A disrupts the NOTCH signaling axis via SNURF/RNF4-mediated ubiquitination of HES1

Angelos Papaspyropoulos[1, 2, 3, *], Andriani Angelopoulou[2, 3], Ioanna Mourkioti[2], Aikaterini Polyzou[2], Daniela Pankova[1], Konstantinos Toskas[1], Simone Lanfredini[1], Anastasia A. Pantazaki[4], Nefeli Lagopati[2, 3], Athanassios Kotsinas[2], Konstantinos Evangelou[2], Eric O'Neill[1, *], Vassilis Gorgoulis[2, 3, 5, 6, 7, *]

[1] *Department of Oncology, University of Oxford, Oxford, UK*
[2] *Molecular Carcinogenesis Group, Department of Histology and Embryology, School of Medicine, National Kapodistrian University of Athens (NKUA), Greece*
[3] *Biomedical Research Foundation, Academy of Athens, Athens, Greece*
[4] *Laboratory of Biochemistry, Department of Chemistry, Aristotle University of Thessaloniki, Thessaloniki 54124, Greece*
[5] *Molecular and Clinical Cancer Sciences, Manchester Cancer Research Centre, Manchester Academic Health Sciences Centre, University of Manchester, Manchester, UK*
[6] *Center for New Biotechnologies and Precision Medicine, Medical School, National and Kapodistrian University of Athens, Athens, Greece*
[7] *Faculty of Health and Medical Sciences, University of Surrey, Surrey, UK*
[*] *Corresponding authors*

Epigenetic inactivation of the RASSF1A tumor suppressor is associated with poor prognosis across all sporadic human malignancies. *RASSF1A* promoter methylation has been correlated with tumor dedifferentiation and aggressive oncogenic behavior. Nevertheless, the underlying mechanism of RASSF1A-dependent tumor dedifferentiation remains elusive. We unravel a novel mechanism showing that RASSF1A directly uncouples the NOTCH-HES1 axis, a key suppressor of differentiation. Interestingly, the crosstalk of RASSF1A with HES1 occurs independently from the signaling route connecting RASSF1A with the Hippo pathway that prevents YAP/TAZ regulation of *POU5F1/OCT4*. At the molecular level, we demonstrate that RASSF1A acts as a scaffold essential for the SUMO-targeted E3 ligase SNURF/RNF4 to target HES1 for degradation. The reciprocal relationship between RASSF1A and HES1 is evident across a wide range of human tumors, depicting the clinical significance of the identified pathway. We show that HES1 upregulation in a RASSF1A-depleted environment renders cells non-responsive to the downstream effects of **γ**-secretase inhibitors (GSIs) which restrict signaling at the level of the NOTCH receptor. Altogether, we report a mechanism through which RASSF1A exerts autonomous regulation of the critical Notch effector HES1, thus classifying RASSF1A expression as an integral determinant of the clinical effectiveness of Notch inhibitors.

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ST28

Modelling the Integrin Linked Kinase (ILK)-Kindlin interaction as a means to regulate tension-dependent integrin endocytosis in *Drosophila*

Katerina M. Vakaloglou[1], Konstantina Kalodimou[1,2], Irini Kagianni[1], Nicholaos Paschalidis[1], Christos Delidakis[2] and Christos G. Zervas[1]*

[1]*Biomedical Research Foundation Academy of Athens, Center of Basic Research, Athens, Greece*
[2]*University of Crete, Department of Biology, Iraklio, Greece*
**e-mail: czervas@bioacademy.gr*

Cells in our bodies experience mechanical forces from their microenvironment that affect their function and homeostasis. When cells sense a critical threshold of tension, they hold tight together and allow tissues to function healthily as a group[1]. In certain diseases, cells lose their adhesive properties and get dissociated, as exemplified in muscular dystrophies. Integrin-based adhesions to the extracellular matrix (ECM) are important sites of mechanotransduction[2]. We recently showed that Integrin-linked kinase (ILK) -a core integrin adhesome component- functions as a mechanosensitive switch of integrin endocytosis and thus strengthens cell-matrix adhesion in the *Drosophila* embryo[3]. Nonetheless, we lack a full understanding of how mechanical force is coupled to integrins to regulate endocytosis and forge adhesion to ECM.

We hypothesize that additional proteins of the integrin adhesome synergistically with ILK regulate integrin endocytosis and thus stabilize cell-ECM adhesion. We currently focus on the Kindlin protein [or *Fermitin* (*Fit*) in *Drosophila*] because it interacts directly with the distal NPxY motif of the integrin β cytoplasmic tail[4]. Interestingly, the same binding motif of the integrin β subunit is shared with the endocytosis regulatory proteins (DAB1, DAB2, DOK1, SNX17 etc)[5], putting forward a competition model between Kindlin and component(s) of the endocytic machinery. We demonstrated that knockdown of both *Fit1* and *Fit2* genes severely impairs integrin-mediated adhesion, without affecting ILK recruitment at muscle attachment sites of the *Drosophila* embryo. This finding implies that specific ILK point mutations which abrogate ILK-Fits interactions will not affect ILK's subcellular localization, but can only reduce Fits stable association with integrin β tail. We currently test the competition model, by engineering specific ILK point mutants both *in vitro* -in S2R+ cultured cells- and *in vivo* and examine integrin endocytosis at the muscle attachment sites.

Our results aim to unravel the molecular mechanism of integrin-mediated mechanotransduction in the whole organism.

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ST29

A double mechanism for the detoxification and assimilation of the toxic phytoproduct L-azetidine-2-carboxylic acid in *Aspergillus nidulans*

Ada Biratsi[1], Alexandros Athanasopoulos[1], Christos Gournas[1] & Vicky Sophianopoulou[1]*
[1] *Microbial Molecular Genetics Laboratory, Institute of Biosciences and Applications, National Centre for Scientific Research, Demokritos (NCSRD), Athens, Greece.*

Plants produce toxic secondary metabolites as defense mechanisms against phytopathogenic microorganisms, competitive neighbor plants and predators. L-azetidine-2-carboxylic acid (AZC), a toxic proline analogue produced by members of the Liliaceae and Agavaciae families, is part of such a mechanism. AZC causes a broad range of toxic, inflammatory and degenerative abnormalities in human and animal cells, while some microorganisms have evolved specialized strategies for AZC resistance. However, the mechanisms underlying these processes are poorly understood. Using *A. nidulans* as a model system we have identified a widespread mechanism for AZC resistance in fungi. Our results showed that filamentous *A. nidulans* is able to not only resist AZC toxicity but also utilize it as a nitrogen source. This assimilation, occurs through the γ-aminobutyric acid (GABA) catabolic pathway and the action of the AzhA hydrolase, a member of a the **h**alo**a**cid **d**ehalogenase-like hydrolase superfamily of detoxifying enzymes (HAD). The detoxification process of AZC, is further assisted by the NgnA acetyltransferase, an Mpr1orthologue of *Saccharomyces cerevisiae*. Heterologous expression of AzhA suppresses AZC sensitivity of *S. cerevisiae* strains. Furthermore, a detailed phylogenetic analysis of AzhA homologues in Fungi, Archaea and Bacteria unravels a widespread mechanism for AZC resistance among several microorganisms, including important human and plant pathogens.

ST30

Bacterial biosynthesis of large combinatorial libraries of cyclic oligopeptides and direct functional screening for discovering novel rescuers of disease-associated protein misfolding

Dafni C. Delivoria[1], Ilias Matis[1], Sean Chia[2], Johnny Habchi[2], Michele Perni[2], Nikoletta Papaevgeniou[1,3], Martin Reczko[4], Niki Chondrogianni[1], Christopher M. Dobson[2], Michele Vendruscolo[2], Georgios Skretas[1]*

[1] *National Hellenic Research Foundation, Athens, Greece*
[2] *University of Cambridge, United Kingdom*
[3] *Friedrich Schiller University of Jena, Germany*
[4] *Biomedical Sciences Research Center "Alexander Fleming", Athens, Greece*

Protein misfolding and aggregation are defining features of a wide range of human conditions, such as Alzheimer's disease, Parkinson's disease and cystic fibrosis, which have been collectively termed protein misfolding diseases (PMDs). The vast majority of these remain to date incurable and impose a very high socio-economic burden on humanity. To address this unmet medical need, we have developed a novel integrated bacterial platform for the discovery of potential therapeutics against PMDs. In this system, *Escherichia coli* cells are genetically engineered in order to perform two simultaneous tasks: (i) produce combinatorial libraries of more than 200 million drug-like, head-to-tail cyclic oligopeptides using protein-splicing technology and (ii) enable the identification of the bioactive cyclic peptides that correct the problematic folding and/or inhibit the aggregation of disease-associated misfolding-prone proteins (MisPs) using a genetic assay that links the folding of the target MisP with a fluorescent phenotype. In this way, the bioactive cyclic peptide hits can be identified in an ultrahigh-throughput manner using flow cytometric cell sorting, thus significantly decreasing the overall cost, time and complexity of early drug discovery for PMDs. Herein we present the implementation of this strategy against a model PMD, Alzheimer's disease, which is associated with the aggregation of the amyloid-β peptide (Aβ42). This procedure resulted in the discovery of more than 400 putative aggregation inhibitors, two of which were further tested in vitro and in vivo and found to potently inhibit the aggregation of Aβ42 at sub-stoichiometric ratios. Finally, through a combination of deep sequencing and site-directed mutagenesis we demonstrate how this system can accelerate the determination of structure-activity relationships and define consensus motifs required for high bioactivity in the discovered molecules.

Chemical Biology

ST31

In vitro fermentation of *Pleurotus eryngii* mushrooms by human Gut Microbiota: anti-genotoxic, metabolomic and meta-taxonomic profiling of the products

Paraschos Christodoulou[1,6], Athina Boulaka[1], Marigoula Vlassopoulou[1,3], Panagiotis Christodoulou[5], Evangelia N. Kerezoudi[3], Georgia Saxami[3], Evdokia K. Mitsou[3], Georgios Koutrotsios[2], Georgios I. Zervakis[2], Adamantini Kyriacou[3], Panagiotis Moulos[4], Maria Zervou[1], Vasiliki Pletsa[1], Panagiotis Georgiadis[1]

[1]*National Hellenic Research Foundation, Institute of Chemical Biology, Athens, Greece*
[2]*Agricultural University of Athens, Laboratory of General and Agricultural Microbiology, Votanikos, Greece*
[3]*Harokopio University, Department of Nutrition and Dietetics, Kalithea, Greece*
[4]*Biomedical Sciences Research Center 'Alexander Fleming', Vari Greece*
[5] *Evaggelismos General Hospital, Department of Internal Medicine, Athens, Greece*
[6] *University of Thessaly, Department of Biochemistry and Biotechnology, Larissa, Greece*

Edible mushrooms are known for their health-promoting properties. They contain bioactive compounds, including polysaccharides, mainly β-glucans, that possess immunomodulatory and anti-cancer activities. Their non-digestible dietary fibers content demonstrates a beneficial effect on intestinal well-being, acting as a substrate for the growth and function of intestinal bacterial populations.

Pleurotus eryngii mushrooms derived from Greek habitats were fermented ***in vitro*** by fecal slurries of elderly asymptomatic volunteers (>60 years old), for 24 hours. Fermentations without any additional carbon source were also carried out and used as negative controls. We examined the fermentation-induced changes in fecal microbiota communities using Next Generation Sequencing of seven out of the nine hypervariable regions of the gene for the 16S rRNA. The primary analysis was conducted using the Ion Reporter Suite, followed by data normalization and statistical analysis using the DEseq2 methodology. Global metabolic profile of fermentation supernatants (FSs) was assessed by ^{1}H NMR spectroscopy and metabolites were assigned by 2D NMR spectroscopy and Metabominer platform. Further identification and quantification were performed on 1D ^{1}H NMR spectra by the ASICS package in R. Quantified metabolites were then subjected to multivariate statistical analysis (PCA, OPLS-DA). Furthermore, the anti-genotoxic properties of FSs were explored in human whole blood cells, obtained from 4 non-smoking volunteers, using Lymphocyte Cytokinesis - block Micronucleus Assay.

Cluster analysis of both metataxonomic and metabolomic data showed a significant cluster separation of PE-treated samples relative to controls. Further statistical analysis revealed that the ***in vitro*** fermentation of PE causes extensive alterations in the relative abundance of different Operational Taxonomy Units (OTUs) as well as in the relative concentrations of FS metabolites. In addition, PE seems to have antigenotoxic properties since FSs were found to protect lymphocytes against the damage induced by Mitomycin C, a known genotoxic agent.

Keywords: P.eryngii, edible mushrooms, genoprotection, metabolomics, meta-taxonomics

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SHORT TALKS 6

Molecular & Cellular Basis of Human Diseases II

ST32

Chronic Stress & Exosomes: key players in progression and diagnosis of Alzheimer's disease

Ioannis Sotiropoulos[1*]
[1]Institute of Biosciences & Applications, NCSR "Demokritos", Athens, Greece

Alzheimer's disease (AD), the leading cause of dementia, affects more than 40 million people worldwide with an estimated annual cost >$600 billion, or 1% of global GDP. Despite the significant progress of the understanding of its neurobiological underpinnings, AD remains a complex disease with no effective treatment and poorly understood risk factors. There is an urgent need for clarification of AD precipitating factors and novel biomarkers that will aid disease early diagnosis and prognosis. Our research work focuses on the understanding of the deleterious effects of chronic stress as a risk factor for Alzheimer's disease (AD) and the role of exosomes (small extracellular vesicles), which have been suggested to contribute to the spread of brain pathology in AD and also emerge as a breakthrough biomarker tool that will serve in early diagnosis, prognosis and overall monitoring of the disease progression. Our studies in experimental animals show the aggravating role of chronic stress in AD neuropathology through the overproduction and accumulation of amyloid peptide β (Aβ) and pathological forms of Tau protein which cause structural and functional brain damage (e.g. synapse loss, atrophy, and neuronal dysfunction) as well memory impairment. We have also recently demonstrated that exposure to chronic stress or high levels of the main stress hormones, glucocorticoids (GC), dysregulate and inhibits endolysosomal pathway and autophagy leading to Tau protein accumulation and secretion via exosomes contributing to the propagation of AD brain pathology. Given that in modern lifestyles, individuals are increasingly exposed to high-stress load, it is clear that understanding the mechanistic interactions between chronic stress and the etiopathogenesis of AD will contribute substantially to both the diagnosis and treatment of the disease.

ST33

Adrenergic stimulation of salivary gland epithelial cells can trigger interleukin-6 production through Endoplasmic Reticulum Stress

Kalliopi Moustaka[1], Athanasios Stergiopoulos[1], Roxane Tenta[1], Stergios Katsiougiannis[1,2], Fotini N. Skopouli[1,3]

[1]*Department of Nutrition and Dietetics, Harokopio University, Athens, Greece*
[2]*Center of Basic Research, Biomedical Research Foundation of the Academy of Athens, Greece*
[3]*Department of Internal Medicine and Autoimmune Diseases, Euroclinic, Athens, Greece*

Sjögren's syndrome (SS) is an autoimmune disease characterized by chronic inflammation of specific secretory epithelia, such as those of salivary glands (SG). Studies have revealed that the salivary gland epithelial cells (SGEC) from SS patients, in culture, retain an activated phenotype probably playing a protagonist role in the initiation of the disease autoimmune lesion. IL-6 expressed by SGEC in SS, is one of the major mediators in the induction and perpetuation of the tissue immune injury.
Accumulated clinical data showed that stress and more specifically chronic, intrinsic (anxious personality) or extrinsic (chronic stressful events), may influence the development and exacerbation of autoimmune diseases. Chronic stress leads to an excess of sympathetic and deficient parasympathetic activation. Noradrenaline is the hormone of the sympathetic system that is up-regulated during stress. Noradrenaline is also the physiologic SGECs stimulus, through β-adrenergic receptors, for protein production and saliva secretion. The central cell organelle, involved in this function, is the Endoplasmic Reticulum (ER) which is characteristically extended in this type of cells. We have previously shown that, in SGEC of SS patients, ER is stressed and beta-adrenergic signaling is enhanced.
The aim of this study was to investigate the effect of adrenergic stimulation on IL-6 production by SGEC and to study the involvement of ER stress on this stimulation pathway. Using primary SGEC lines derived from SS patients and controls SG biopsies, the beta-adrenergic receptors were stimulated with epinephrine and IL-6 mRNA expression was assessed by qRT-PCR. Our data showed that adrenergic stimulation significantly upregulated IL-6 in SGEC in patients as well as in controls. Alleviation of ER stress using Tauroursodeoxycholic acid (TUDCA), prevented IL-6 upregulation. Our findings suggest that adrenergic stimulation of SGEC contributes to IL-6 induction in an ER stress-dependent manner.

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ST34

Anaplastic Lymphoma Kinase inhibition as an effective treatment strategy against the enhanced lung carcinogenesis and angiogenesis related to decreased PTPRZ1 expression

Despoina Ntenekou[1*], Pinelopi Kastana[1], Eleni Mourkogianni[1], Mihaela-Karina Enake[1], Sophia Nikou[2], Gonzalo Herradón[3], Eleni Papadaki[2], Evangelia Papadimitriou[1]

[1]Laboratory of Molecular Pharmacology, Department of Pharmacy, University of Patras, Greece
[2]Department of Anatomy, School of Medicine, University of Patras, Patras, Greece
[3]Departamento de Ciencias Farmacéuticas y de la Salud, Facultad de Farmacia, Universidad San Pablo-CEU, CEU Universities, Madrid, Spain

Anaplastic lymphoma kinase (ALK) is a tyrosine kinase (TK) receptor that has been correlated with oncogenesis due to ALK TK activation or expression of abnormal fusion constitutively active ALK proteins. Limited knowledge exists related to soluble ALK ligands and their role in ALK activation in health or disease. It has been previously suggested based on *in vitro* data that receptor protein tyrosine phosphatase zeta 1 (PTPRZ1) regulates ALK TK activity but the exact pathway involved, or the functional significance of such potential interaction have not been elucidated. In the present work, we show that ALK TK inhibitors abolish the enhanced lung carcinogenesis and angiogenesis observed in *Ptprz1*$^{-/-}$ mice, with minimal effect on *Ptprz1*$^{+/+}$ mice. ALK phosphorylation and downstream signaling is enhanced in *Ptprz1*$^{-/-}$ lung microvascular endothelial cells (LMVEC) or in human umbilical vein endothelial cells (HUVEC) following inhibition of PTPRZ1 tyrosine phosphatase activity. ALK TK inhibitors abolish the enhanced angiogenic properties of both LMVEC and HUVEC following genetic or pharmacological inhibition of PTPRZ1. Moreover, they inhibit $VEGFA_{165}$ induced HUVEC proliferation and migration that is known from our previous studies to depend on PTPRZ1. Finally, ALK TK inhibitors abolish the mitochondrial elongation observed in *Ptprz1*$^{-/-}$ compared to *Ptprz1*$^{+/+}$ LMVECs or in HUVEC following treatment with $VEGFA_{165}$ or a PTPRZ1 tyrosine phosphatase inhibitor. Using numerous pharmacological inhibitors of the PTPRZ1-related signaling pathway in HUVEC, we found that ALK lays downstream of PTPRZ1 in the VEGFA-activated pathway, and together with $\alpha_v\beta_3$ integrin are required for regulation of mitochondrial elongation and endothelial cell proliferation and migration. Collectively, our data suggest that ALK regulates angiogenesis *in vitro* and *in vivo* and lung carcinogenesis *in vivo* and ALK TK inhibitors could be an effective therapeutic strategy in cancer or other pathologies with excessive angiogenesis related to decreased PTPRZ1 expression or tyrosine phosphatase activity.

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ST35

Amplification of stable tau fibril conformers in cell-based systems

Taxiarchis Katsinelos[1,2*], Eleni Dimou[2,3], Manuel Schweighauser[1], David Klenerman[2,3], Michel Goedert[1], William A. McEwan[2*]

[1] *MRC Laboratory of Molecular Biology, Cambridge, United Kingdom*

[2] *Department of Clinical Neurosciences, UK DRI at the University of Cambridge, Cambridge, United Kingdom*

[3] *Yusuf Hamied Department of Chemistry, University of Cambridge, Cambridge, United Kingdom*

**e-mail: katsinelos@mrc-lmb.cam.ac.uk, wm305@cam.ac.uk*

The assembly of the tau protein into filaments occurs in numerous neurodegenerative diseases, including Alzheimer's disease[1]. Recent advances in cryo-electron microscopy have yielded high-resolution structures of brain-derived tau filaments, which seem to adopt unique conformations in different diseases[2–4]. Consequently, major questions regarding tau pathology remain to be addressed in detail: Does the seeded aggregation of tau contribute to the inheritance of disease-specific conformers? Which species are involved in the propagation of tau pathology? How is the nature of the filament structure related to disease pathogenesis?

Here, we treated our previously established full-length tau seeded aggregation cellular system[5] with a range of recombinant and brain-derived tau filaments. These mixed populations were subjected to limited dilution that allowed us to isolate clonal cells that stably propagate seeded aggregated tau over several passages. Additionally, we combined a series of biochemical, biophysical, and structural assays for the detailed characterization of these unique tau conformers. Finally, we verified our findings by propagating these *in cellulo*-amplified filament structures in murine primary neuronal cultures.

Our findings suggest that the cellular propagation of diverse tau fibrils is supported and that unique conformations are stable following re-introduction to cells. In contrast, we observe that conformational fidelity is rapidly lost during *in vitro* templated aggregation, suggesting the presence of cell-specific mechanisms that are essential for the faithful replication of tau filament structures. Most importantly, we provide evidence that the tau fibrils derived from each clonal population are unique and their properties resemble the ones from the original source of seeds that was amplified inside cells.

Taken together, our work raises the prospect that disease-origin tau conformations can be propagated near-infinitely in cell-based systems. Elucidating the structure-function relationships of tau fibrils is critical to a better understanding of neurodegeneration and, ultimately, to develop novel clinical therapies.

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SHORT TALKS 7

Regulation of Gene Expression & Epigenetics II

ST36

The metabolic enzyme pyruvate dehydrogenase phosphatase 1 (PDP1) enhances HIF activity under hypoxia by an acetylation-dependent mechanism

Angeliki Karagiota[1,2], Amalia Kanoura[1], George Simos[1], Efrosyni Paraskeva[2] and Georgia Chachami[1]
[1]Laboratory of Biochemistry and [2]Laboratory of Physiology, Faculty of Medicine, University of Thessaly, Biopolis 41500, Larissa, Greece

Cancer cells respond to low oxygen by activating the "hypoxia response pathway", which mainly entails activation of a family of transcriptional activators known as the Hypoxia-inducible factors (HIF). HIF-1, the most well studied member, activates the transcription of numerous genes involved in metabolic reprogramming of cancer cells. In addition to its oxygen-dependent expression, HIF-1 is also regulated by phosphorylation. Although several kinases mediating HIF-1α phosphorylation have been identified, the role of phosphatases in the regulation of HIF-1 is poorly characterized. To address this issue, we analyzed HIF activity in cells in which expression of the catalytic subunits of known human phosphatases was silenced, using a siRNA library[1]. We discovered Pyruvate Dehydrogenase Phosphatase 1 (PDP1) as a regulator of HIF-1 transcriptional activity. PDP1 is a key mitochondrial metabolic enzyme that dephosphorylates and activates Pyruvate Dehydrogenase (PDH), leading to increased conversion of pyruvate into acetyl-CoA. Inactivation of PDH by phosphorylation is catalyzed by PDK1, expression of which is regulated by HIF-1. Silencing of PDP1 significantly downregulated HIF mediated transcription of a hypoxia reporter gene and the expression of HIF-1-dependent genes, including PDK1, in HeLa cells. Accordingly, PDP1 overexpression or its hormonal stimulation by insulin enhanced HIF activity under hypoxia. The PDP1-mediated positive effect on HIF-1 did not involve physical interaction of PDP1 with HIF-1α but entailed increased binding of HIF-1 to target promoter chromatin. Treatment of PDP1-silenced cells with acetate or with the histone deacetylase (HDAC) inhibitor trichostatin A led to recovery of HIF-1 activity, suggesting that the PDP1 effects on HIF-1 are mediated by alterations in protein acetylation levels. Overall, our data suggest the operation of a homeostatic metabolic circuit between the mitochondria and the nucleus involving PDP1, HIF-1 and PDK1. These data could provide valuable insights into the use of HIFs as anti-cancer therapeutics targets.

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ST37

Stochastic activation of the "MMP9 protein network" is required for cellular reprogramming towards pluripotency

Eleftheria Klagkou[1], Dimitrios Valakos[1], Ioanna Polydouri, Georgios Sianidis, Giorgos Panagopoulos, Giannis Vatsellas, Marios Agelopoulos, and Dimitris Thanos*
Biomedical Research Foundation Academy of Athens, Athens, Greece
[1]These authors contributed equally
**Corresponding author. Tel: +30 2106597244; E-mail: thanos@bioacademy.gr*

Cellular reprogramming towards pluripotency via the overexpression of the OSKM (Oct4, Sox2, Klf4 and cMyc) factors is a stochastic process which requires global alterations of the epigenome and genome function. Previous studies from our laboratory have shown that OSKM orchestrate the gradual and stochastic assembly of a gene regulatory network in a remarkably small number of cells, the 9TR GRN, composed of 9 transcriptional regulators (Cbfa2t3, Gli2, Irf6, Nanog, Ovol1, Rcan1, Taf1c, Tead4 and Tfap4) which is required for cellular reprogramming. To study the dynamic biochemical alterations occurring in cells during their route to pluripotency, we generated GFP reporters bearing the enhancers of Irf6 and Ovol1 and isolated the rare Irf6 and Ovol1 expressing cells. In parallel, we identified novel reprogramming specific enhancer elements (RSE) bound by OSKM at high density. We demonstrated that GFP+ cells representing the Irf6, Ovol1 and RSE expressing cells possess a higher reprogramming efficiency when compared to GFP- cells. These data suggest that this population of cells is committed to reprogramming. RNA-seq analysis revealed high-level expression of genes involved in Mesenchymal to Epithelial Transition (MET). Importantly, these cells express sets of genes encoding proteins participating in overlapping networks involved in extracellular matrix organization. The central node of these networks is the matrix metallopeptidase 9 (MMP9), which we showed that it is regulated by direct binding of both Ovol1 and Irf6 to its enhancer element. Pharmacological inhibition of MMP9 enzymatic function during fibroblast reprogramming dramatically decreased their reprogramming efficiency, while it had no effect on the reprogramming of hepatocytes, which does not require MET. In summary, we identified an 9TR-GRN-orchestrated "MMP9 network" required for cellular reprogramming. We hypothesize that the function of the MMP9 network induces detachment of cells from the extracellular matrix to establish cell-cell junctions, thus to acquire the epithelial state, which is a critical process for the reprogramming of fibroblasts to pluripotency.

ST38

CRISPR editing combined with RNA-seq and CLIP-seq analysis reveals novel cellular functions of an RNase Z paralogue in noncoding RNA biogenesis

Athanasios-Nasir Shaukat[1], Mahmoud F. Dondeti[2], Eleni G. Kaliatsi[1], Ilias Skeparnias[1,3], Anastasios Vourekas[2], Constantinos Stathopoulos[1*]

[1]*Department of Biochemistry, School of Medicine, University of Patras, 26 504 Patras, Greece*
[2]*Department of Biological Sciences, Louisiana State University, Baton Rouge, LA 70803, USA*
[3]*Laboratory of Molecular Biology, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD 20892, USA*
[*]*e-mail:* cstath@upatras.gr

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 MALAT1[1], knowledge on the biological role of RNase Z^S, which is found in the cytoplasm is limited[2,3]. To elucidate its role we knocked out *ELAC1* in HEK293T cells. The edited cells exhibited reduced proliferation and motility, vulnerability to serum deprivation, higher resistance to glucose deprivation, increased mitochondrial mass per cell and overall altered morphology when compared to the wild type cells. Subsequent NGS analysis showed an overall downregulation of important genes implicated in the same processes and rescue experiments followed by whole transcriptome sequencing revealed the differential expression of important classes of ncRNAs such as snoRNAs, miRNAs, tRNAs and tRNA-derived fragments. To elucidate the substrate repertoire of RNase Z^S we performed CLIP-seq analysis using the WT enzyme and a catalytic null mutant. The analysis unveiled interactions not only with tRNAs, but also with snoRNAs and other important ncRNAs such as *RN7SK*, suggesting an important role of RNase Z^S in ncRNA biogenesis that is reported for the first time. The current study provides evidence that the role of RNase Z^S is not limited to tRNA repair and might be also involved in snoRNA turnover or the assembly of important ribonucleoprotein complexes.

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DNA damage/repair

ST39

The RAD52-independent Alternative Lengthening of Telomeres in neoplasia is mediated by intra-chromosomal conservative Break-Induced Replication

Eleni Dragona, Veronica Cherdyncheva, Chara Papalouka, Maria Kyriakaki, Sarantis Gagos
Biomedical Research Foundation of the Academy of Athens, Greece

Telomeres protect the ends of linear chromosomes from degradation, DNA damage checkpoint activation and illegitimate recombination. Telomere integrity ensures genomic stability and protects from carcinogenesis, whereas continuous proliferation and survival of cancer cells is sustained by the activation of telomere length maintenance mechanisms. Most human malignancies endure growth-permissive telomere length, by activating the reverse transcriptase telomerase, while 10-15% (including highly aggressive mesenchymal tumors) engage the Alternative Lengthening of Telomeres (ALT). Our team showed for the first time, that in human neoplasia, ALT is operated by a conservative DNA repair process, known as Break-Induced Replication (BIR). Recent studies revealed that ALT telomere neo-synthesis takes place via two distinct BIR pathways that depend or not, on the recombinase RAD52. It is currently unknown, if these two types of ALT act synergistically or if they counteract each other. Moreover, it is not clear if they imply intra-chromosomal or inter-chromosomal telomeric recombination. To answer these questions and to identify key components of RAD52-independent ALT, we developed a modification of Chromatid-Orientation Fluorescent in Situ Hybridization, capable to quantify conservative intra-chromosomal BIR. We applied this potent technology along to a whole arsenal of telomere functionality assays, in human telomerase positive or ALT+ cell lines exposed to DNA damage response inhibitors and/or RNA silencing of major components of Homologous Recombination and in human osteosarcoma ALT+ U2OS cells knocked-out for RAD52 via CRISPR-Cas9, conditionally overexpressing Cyclin-E to recapitulate oncogene-induced replication stress. Our data reveal that RAD52 suppresses intra-chromosomal BIR-mediated ALT, suggesting that the RAD52-independent telomere lengthening is mostly operated by intra-chromosomal homologous recombination and relies on the replication fork regressor SMARCAL1 and the fork remodeler MUS81. Our results provide key insights into the biological mechanisms operating in telomerase-independent telomere lengthening identifying putative targets for ALT suppressors with potential diagnostic or therapeutic value.

ST40

The Fanconi Anaemia pathway contributes to survival of cells undergoing aberrant DNA licensing

Nibal Badra[1], Ourania Preza[1], Rami Mäkelä[2], Juha K. Rantala[2], Stavros Taraviras[3] and Zoi Lygerou[1*]
[1]Department of General Biology, School of Medicine, University of Patras, Greece.
[2]Misvik Biology, Turku, Finland
[3]Department of Physiology, School of Medicine, University of Patras, Greece
**Corresponding author*

Genome duplication and chromosome segregation constitute the cornerstones of a dividing somatic cell. DNA licensing is a conserved mechanism ensuring that a full copy of the genetic material is produced whereby restricting single origin firing to once-per-cell-cycle. Licensing of already fired origins induces re-replication, a substrate of extensive replication stress and DNA damage. Interestingly, abnormal expression of licensing factors is found in diverse cancer types, and associates to increased genomic instability and adverse prognosis. Deregulated licensing represents a threat to genome integrity with potential relevance in targeted-therapy, yet the pathways responsible for signaling re-replication and the molecular mechanisms responding to this phenomenon remain poorly understood. To unveil candidate pathways fostering cell survival upon induced re-replication, we performed a high content screening in non-transformed cells over-expressing Cdt1 or defective in the expression of the licensing inhibitor Geminin. We identified a previously uncharacterized role of the Fanconi Anaemia pathway in signaling replication defects from cells undergoing origin re-licensing. Activation of Fanconi Anaemia signaling was evidenced by formation of FANCD2 foci early during re-replication, indicating regions of halted fork progression partially distinct from double strand break sites. Loss of FANCD2 in Geminin-depleted cells increased fork stalling and double strand breaks, with damaged cells arresting in G2 and ultimately undergoing cell death. At the mechanistic level, our data suggest that FANCD2 prevents unrestrained DNA synthesis upon Geminin loss. Specific compounds previously shown in our group with the potential to impaired DNA licensing in FANCD2-deficient cancers could provide a novel therapeutic strategy to target cancer cell survival.

SHORT TALKS 8

Molecular & Cellular Basis of Human Diseases III

ST41

Silencing of *Foxo1* gene in the livers of APOE*3L.CETP mice affects the expression of genes related to glucose metabolism and improves glucose tolerance

Dimitris Nasias[1,2], Jan Freark de Boer[3,4] Niels L. Mulder[3], Vasiliki Theodorou[5], Jan Albert Kuivenhoven[3], Dimitris Kardassis[1,2,*]

[1]*Laboratory of Biochemistry, Division of Basic Sciences, University of Crete Medical School, Heraklion, Greece*

[2]*Gene Regulation and Genomics group, Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology of Hellas, Heraklion, Greece*

[3]*Department of Pediatrics, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands.*

[4]*Department of Laboratory Medicine, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands*

[5]*Genomics Facility, Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology-Hellas, Heraklion, Greece*

**Corresponding author: kardasis@imbb.forth.gr*

Aims: Metabolic syndrome (MetS) is a cluster of conditions associated with abdominal obesity that predispose to cardiovascular disease. Development of insulin resistance and imbalance in glucose metabolism are crucial for MetS progression. In the present study, we aimed to investigate whether silencing of the gene encoding the insulin-regulated transcription factor FOXO1 in the liver of APOE*3L.CETP mice impacts MetS pathogenesis.

Methods: Adult male APOE*3L.CETP mice were intravenously injected with an AAV8 targeting the endogenous *Foxo1* gene (AAV8-shFoxo1) or a Control virus and were subsequently fed a high-fat diet (HFD) for 3 months. Body weights, lipid profiles, glucose levels, glucose and insulin tolerance were monitored. Hepatic transcriptome profiling was obtained by RNAseq.

Results: Hepatic *Foxo1* mRNA and protein levels were substantially reduced (-70% and -40% respectively, $p<0.05$) in mice injected with shFoxo1 compared to control. This however did not translate into differences in fasted blood glucose and serum lipid levels. Importantly, marked improvement in glucose tolerance ($p<0.01$) and a trend towards improved insulin sensitivity was apparent in shFoxo1-injected mice. RNA sequencing showed that genes related to FOXO1 signaling, PI3K-Akt signaling and insulin resistance were downregulated, whereas genes related to oxidative phosphorylation were upregulated. In silico analysis of published ChIP-seq FOXO1 binding events combined with our RNAseq data confirmed that FOXO1 transcriptionally regulates genes involved in cholesterol metabolism and insulin signaling.

Conclusions: Down-regulation of pathways such as gluconeogenesis, glucose production and glucose uptake combined with increased Oxidative Phosphorylation by shFoxo1 could contribute to the improvement of glucose tolerance in HFD-fed APOE*3L.CETP mice.

ST42

The role of RANKL in mammary epithelial cell proliferation, expansion and progesterone-driven carcinogenesis

Anthi Kolokotroni[1,2], Vasiliki Geronta[1], Evi Gkikopoulou[1,2], Vagelis Rinotas[2], Danae Zareifi[4], Maritina Rouchota[5], Ilias Lymperopoulos[6], Leonidas Alexopoulos[4], George Loudos[5], Maria Denis[3], Niki Karagianni[3], Eleni Douni[1,2]

[1]Department of Biotechnology, Agricultural University of Athens, Iera Odos 75, 11855, Athens, Greece
[2]Institute for Bioinnovation, B.S.R.C. "Alexander Fleming", Fleming 34, 16672, Vari, Greece
[3]Biomedcode Hellas SA, Fleming 34, 16672, Vari, Greece
[4]Department of Mechanical Engineering, National Technical University of Athens, Athens, Greece
[5]Bioemission Technology Solutions (BIOEMTECH), Lefkippos Attica Technology Park, NCSR "Demokritos", Ag. Paraskevi-Athens, Greece
[6]1rst Breast Clinic, Iaso Hospital, 37-39 Kifissias, 15123 Maroussi, Greece

Receptor activator of nuclear factor-κB ligand (RANKL), is induced by progesterone and is critically involved in the proliferation of mammary epithelial cells (MECs) through paracrine signaling, playing a crucial role in mammary gland development and breast carcinogenesis. To further investigate the role of RANKL overexpression in breast pathophysiology, we performed a series of analyses in mammary glands of osteoporotic transgenic mice expressing human RANKL (TgRANKL), including molecular and protein expression of RANKL and other markers, the profile of epithelial populations, the density of the epithelial ductal tree, and the susceptibility to carcinogenesis. Our results demonstrated expression of human RANKL at the luminal epithelial cells that was further upregulated upon synthetic progesterone (MPA) administration as quantified by qPCR. We also identified epithelial expansion in the mammary glands of TgRANKL mice compared to WT that was reversed by the RANKL inhibitor Denosumab (Dmab). Immunofluorescence analysis revealed increased epithelial cell proliferation as shown by Cyclin D1 staining and expansion of mammary epithelial stem cells through flow cytometry. RNA sequencing analysis confirmed increased cell proliferation and identified deregulated gene expression and activation of signaling molecules associated with carcinogenesis. To investigate the incidence and progression of carcinogenesis in TgRANKL mice, we established an MPA-induced breast cancer model in humanized TgRANKL mice, in the presence of prophylactic treatment with Denosumab. Our results showed a significant attenuation of carcinogenesis through pharmaceutical RANKL inhibition (Dmab). RNA sequencing analysis of adjacent mammary glands showed that RANKL inhibition through Dmab affected the expression levels of a plethora of pathways and molecular indicators of breast carcinogenesis. Collectively, our results demonstrated that overexpression of RANKL in the mammary glands increased mammary density, which is a risk factor for breast cancer, while preventive therapeutic inhibition of RANKL attenuated the incidence of breast cancer.

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ST43

Prox1 inhibits c-Myc dependent gene regulatory program and metabolic pathway to suppress the proliferation of breast cancer cells

Artemis Michail[1*], Dimitris Gkikas[1], Valeria Kaltezioti[1] and Panagiotis K Politis[1]
[1] *Center for Basic Research, Biomedical Research Foundation of the Academy of Athens, 4 Soranou Efesiou, 115 27, Athens, Greece*

Breast cancer is one of the most lethal malignancies in women. Despite the progress in tumor biology and provided therapies, breast cancer is characterized by rapid growth and low survival rates. These observations underscore the need for novel insights in the molecular mechanisms of malignant transformation and progression. To this end, here we identify Prox1 as a transcription factor with a critical role in suppressing malignant transformation in breast cancer. In particular, our meta-analysis of the expression data from Oncomine (www.oncomine.org) and TCGA (The Cancer Genome Atlas, www.cancergenome.nih.gov) showed that Prox1 is dramatically reduced in breast tumors due to epigenetic silencing. In addition, we provide functional evidence that Prox1 strongly suppresses the proliferation and migration of human breast cancer cell lines in a non-apoptotic way. Consistently, xenogarft assays in NOD/SCID mice indicate that Prox1 is sufficient to suppress tumor growth *in vivo*. Interestingly, Prox1 induces a gene expression program characterized by down-regulation of c-Myc and c-Myc-regulated genes (including *Glut1*, a glucose transporter, *HK2*, the enzyme that catalyzes the rate-limiting step of glycolysis, and *PDK1, the* glycolysis gatekeeper) as well as induction of *Mpc1,* the mitochondrial pyruvate carrier. These data suggest that Prox1 may inhibit the establishment of Warburg effect in breast cancer cells. To further study this hypothesis, we will perform metabolic assays in breast cancer cells. We are also going to investigate the ability of Prox1 to suppress tumor growth *in vivo* in a disease-relevant environment by performing orthotopic xenografts in NOD/SCID mice. In summary, our study establishes Prox1 as a breast tumor inhibiting factor via negative regulation of c-Myc and glycolysis, and potential therapeutic target in breast cancer.

ST44

Protein network and pathway analysis in a pharmacogenetic study of cyclosporine treatment response in Greek patients with psoriasis

Eleana F. Stavrou[1,2], Charis Antonatos[1], Evangelos Evangelou[3,4], Andreas Liaropoulos[1], Danai Digka[1], Efterpi Zafiriou[5], Angeliki Roussaki-Schulze[5], Aikaterini Patsatsi[6], Dimitris Sotiriadis[6], Sofia Georgiou[7], Nicholas Moschonas[2,8], Yiannis Vasilopoulos[1*]

[1]Laboratory of Genetics, Section of Genetics, Cell Biology and Development, Department of Biology, University of Patras, Greece,
[2]Molecular Genetics Unit, Lab. Of General Biology, Medical School, University of Patras, Greece,
[3]Department of Hygiene and Epidemiology, Medical School, University of Ioannina, Greece,
[4]Department of Epidemiology & Biostatistics, MRC Centre for Environment and Health, Imperial College London, UK,
[5]Department of Dermatology, University General Hospital Larissa, University of Thessaly, Greece,
[6]2nd Dermatology Department, Medical School, Papageorgiou Hospital, Aristotle University, Greece,
[7]Dermatology Department, Medical School, University of Patras, Greece,
[8]Foundation of Research & Technology, Institute of Chemical Engineering Science (ICE-HT),
**e-mail: iovasilop@upatras.gr*

Psoriasis is a chronic, inflammatory skin disease that affects 2-3% of the population worldwide. Although cyclosporine is a well-established systemic therapy for moderate to severe psoriasis, patients show important heterogeneity (~70%) in their response to treatment. The aim of our study was the pharmacogenetic analysis of Greek patients with psoriasis based on the construction of the cyclosporine-pathway Protein-Protein Interaction (PPI) network. The cyclosporine related protein interactome was constructed through the PICKLE meta-database (**www.pickle.gr**) [1]. Based on this, we selected 30 single nucleotide polymorphisms mapped on 22 of the key molecular nodes in the cyclosporine signaling cascade, filtered through their functional significance and/or MAF≥5%. Forward, reverse and the appropriate iPLEX extension PCR primers were designed using the Assay Design Suite (ADS) software. SNP genotyping of 96 patients with psoriasis treated with cyclosporine for at least 3 months was performed through the iPLEX®GOLD panel of the MassARRAY®System (Agena Biosciences). In addition to single-SNP analysis carried out in Stata 13.1, haplotypes were also constructed with the Hapstat 3.0 software [2]. The mean age of the patients included was 46.86 years, while 67 had a positive response to therapy (69.79%; ΔPASI≥75%). Our single-SNP analyses showed statistically significant associations between *PPP3R1* rs1868402 (*P*=0.02) and *MALT1* rs2874116 (*P*=0.03) polymorphisms with positive response to cyclosporine therapy, but also a trend for association for other 9 genetic biomarkers (*JAK2* rs10758669, *PP3CC* rs11780915, *PTGIR* rs12461917, *CALM1* rs12885713, *SLC8A1* rs13017846, *NFKBIA* rs17103265, *CARD11* rs17834873, *IPO9* rs8024 and *PPI* rs8177826). Haplotype analysis further enhanced the predictive value of rs1868402 as a pharmacogenetic biomarker for cyclosporine therapy (*P* = 0.0197). Our findings have the potential not only to improve our prognosis of cyclosporine therapy in psoriasis patients but more importantly to be applied as a methodological approach in the pharmacogenetics of biological therapies in complex diseases.

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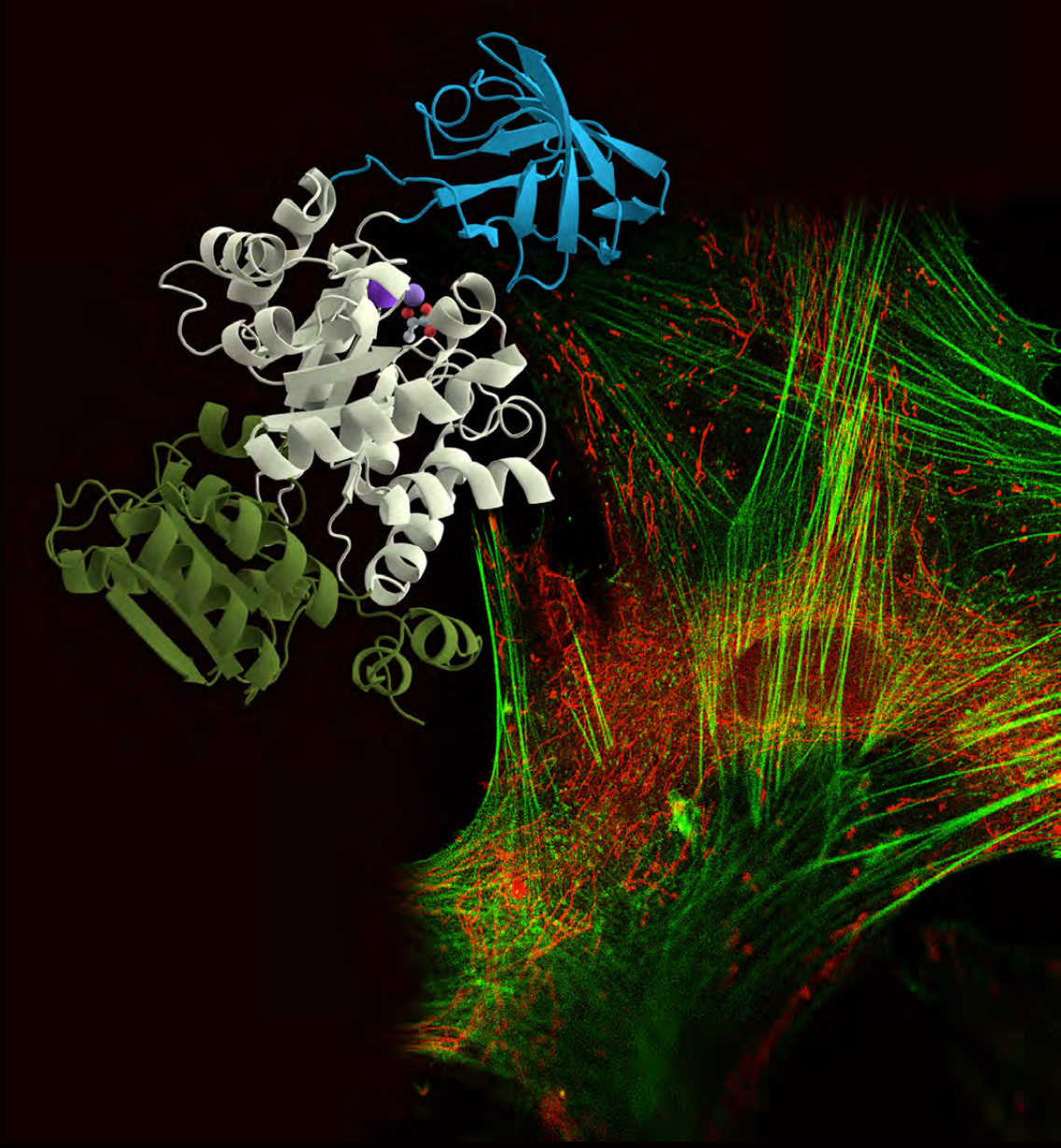
EEBMB



e-posters_

71 Πανελλήνιο Συνέδριο
ΕΛΛΗΝΙΚΗΣ ΕΤΑΙΡΕΙΑΣ
ΒΙΟΧΗΜΕΙΑΣ και ΜΟΡΙΑΚΗΣ ΒΙΟΛΟΓΙΑΣ

P1

Differential dose- and tissue-dependent effects of foxo on aging, metabolic and proteostatic pathways

Maria S. Manola*, Sentiljana Gumeni, and Ioannis P. Trougakos
Department of Cell Biology and Biophysics, Faculty of Biology, University of Athens, Panepistimiopolis, Athens 15784, Greece

Aging is the gradual deterioration of physiological functions that culminates in death. Several studies across a wide range of model organisms have revealed the involvement of FOXO (forkhead box, class O) transcription factors in orchestrating metabolic homeostasis, as well as in regulating longevity. To study possible dose- or tissue dependent effects of sustained *foxo* overactivation we utilized two different *Drosophila* transgenic lines expressing high and relatively low *foxo* levels and overexpressed *foxo* either ubiquitously or in a tissue-specific manner. We found that ubiquitous *foxo* overexpression (OE) accelerated aging, induced the early onset of age-related phenotypes, increased sensitivity to thermal stress and deregulated metabolic and proteostatic pathways; these phenotypes were more intense in transgenic flies expressing high levels of *foxo*. Interestingly, there is a defined dosage of *foxo* OE in muscles and cardiomyocytes that shifts energy resources into longevity pathways and thus ameliorates not only tissue but also organismal age-related defects. Further, we found that *foxo* OE stimulates in an Nrf2/cncC dependent-manner counteracting proteostatic pathways, e.g., the ubiquitin-proteasome pathway, which is central in ameliorating the aberrant *foxo* OE-mediated toxicity. These findings highlight the differential dose- and tissue-dependent effects of foxo on aging, metabolic and proteostatic pathways, along with the foxo-Nrf2/cncC functional crosstalk.

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P2

Systematic mutational analysis of the Sec24 cargo-binding sites involved in COPII-dependent ER-exit of membrane proteins

Georgia Maria Sagia[1], Mariangela Dionysopoulou[1] & George Diallinas[1,2]
[1]Department of Biology, National and Kapodistrian University of Athens, Panepistimioupolis, 15784 Athens, Greece
[2]Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology, 70013 Heraklion, Greece

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 prior to their trafficking[1]however, remain about the organization of the ER-Golgi interface in cells and the type of membrane structures mediating traffic from ERES. To investigate these, we use transgenic tagging in Drosophila flies, 3D-structured illumination microscopy (SIM and pack into coat protein complex-II (COPII) secretory vesicles, which are destined to fuse with the *cis*-Golgi[2,3]. Following Golgi maturation, membrane proteins exit from the *trans*-Golgi network (TGN) in clathrin coated vesicles which are directed to the PM[3,4,5]. However, our recent findings, using the fungus *Aspergillus nidulans* as a model eukaryotic system, showed that several transmembrane cargoes follow an *unconventional* sorting pathway that bypasses the Golgi, which in turn suggests the existence of distinct *cargo-specific COPII subpopulations*[6,7]. Selective recruitment of membrane cargoes by Sec24, an essential subunit of the COPII coat, is mediated by direct or indirect interactions between cargo-binding sites on Sec24 and specific sorting motifs within cargo proteins[8]. Here we investigate the molecular basis of Sec24 interactions with specific cargoes, especially those that bypass the Golgi (e.g., nutrient transporters). In particular, we develop a controllable system to repress the synthesis of the endogenous Sec24 protein, and use it to investigate the functional effect of systematically designed Sec24 mutations carried in a plasmid vector that is introduced by standard reverse genetics of *A. nidulans*. Our results show that amino acid substitutions in conserved or semi-conserved residues located in the A and B cargo-binding sites of Sec24 lead to differential ER-retention of specific membrane cargoes.

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P3

Protein binding properties of oxime carbamate derivatives

Vasileios Vrazas [1], Nefeli Sotiraki[1], Anastasios Panagopoulos[1], Konstantina Fylaktakidou[1,2], Katerina R. Katsani[1*]

[1]Laboratory of Biochemistry and Molecular Virology, Department of Molecular Biology and Genetics, Democritus University of Thrace, University Campus, Dragana, 68100 Alexandroupolis, Greece.
[2]Laboratory of Organic Chemistry, Chemistry Department, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece

Oxime derivatives are organic compounds which exhibit a variety of anticancer, antimicrobial, antioxidant and anti-inflammatory activities.[1] They possess the structural fragment R^1R^2-C=N-OR^3 which is susceptible to N-O bond homolysis, a characteristic that leads to useful synthetic applications via the R^1R^2-C=N· radical. Furthermore, a number of publications have indicated that especially the aroyloxyl radicals (R^3=Ar-CO) derived from compounds which exhibit high affinity to DNA, are able to photo-cleave DNA with a reactivity similar to hydroxyl radicals.[2] Recently, we have investigated the ability of ***O***-carbamoyl oximes (or oxime carbamates) to photo-cleave DNA.[3] Interestingly, although all derivatives have shown affinity to DNA and were able to give N-O homolysis under irradiation, only the ***p***-halogenated ones have shown specificity in DNA photo-cleavage. We have decided to expand our studies and investigate the association of these compounds with the biologically abundant proteins albumin and lysozyme.[4] We postulate that a putative protein photo-cleavage activity may lead to the use of such compounds to a broader spectrum of applications, whereas binding to proteins in the absence of photo-reactivity may assure a more specific activity only to DNA and uses in cancer photo-therapy. Therefore, we employed fluorescence spectroscopy and studied the ability of the most active derivatives (towards DNA) to bind to proteins based on the quenching of tryptophan fluorescence in their presence in solution. Overall, the tested compounds have exhibited quite good binding properties to at least one of the proteins, whereas they did not seem to cause any protein photo-damage.

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P4

The tumor-promoting phenotype of ionizing radiation-induced senescent human breast stromal fibroblasts is complemented by the down-regulation of the proteoglycan decorin

Eleni Mavrogonatou[1], Adamantia Papadopoulou[1], Asimina Fotopoulou[1], Stathis Tsimelis[1], Heba Bassiony[2], Andreas M. Yiacoumettis[3], Petros N. Panagiotou[4], Harris Pratsinis[1] and Dimitris Kletsas[1,*]

[1] Laboratory of Cell Proliferation and Ageing, Institute of Biosciences and Applications, National Centre for Scientific Research "Demokritos", 15341 Athens, Greece

[2] Department of Zoology, Faculty of Science, Cairo University, 12613 Giza, Egypt

[3] Plastic and Reconstructive Surgery Department, Metropolitan General Hospital, 15562 Athens, Greece

[4] Department of Plastic Surgery and Burns Unit, KAT General Hospital of Athens, 14561 Athens, Greece

** Correspondence: dkletsas@bio.demokritos.gr*

Among the numerous different types of cancer, breast cancer is generally regarded as the most common malignancy, predominantly in female patients. Beyond the genetic alterations obtained by neoplastic cells, the critical role of the stromal components in cancer development has thus far greatly appreciated. Down-regulation of the small leucine-rich proteoglycan decorin in the stroma is considered a poor prognostic factor for breast cancer progression. Ionizing radiation, an established treatment for breast cancer, provokes the premature senescence of the adjacent to the tumor stromal fibroblasts. Here, we showed that senescent human breast stromal fibroblasts are characterized by the down-regulation of decorin at the mRNA and protein level, as well as by its decreased deposition in the pericellular extracellular matrix *in vitro*. Senescence-associated decorin down-regulation is a long-lasting process rather than an immediate response to γ-irradiation. Growth factors were demonstrated to participate in an autocrine manner in decorin down-regulation, with bFGF and VEGF being the key mediators of the phenomenon. Autophagy inhibition by chloroquine reduced, while autophagy activation using the mTOR inhibitor rapamycin enhanced decorin mRNA levels, respectively. Interestingly, the secretome from a series of untreated and irradiated human breast cancer cell lines with different molecular profiles inhibited decorin expression in young and senescent stromal fibroblasts, which was annulled by SU5402, a bFGF and VEGF inhibitor. The novel phenotypic trait of senescent human breast stromal fibroblasts revealed here is added to their already described cancer promoting phenotype characterized by the enhanced expression and enzymatic activity of ECM-degrading proteases, the down-regulation and decreased biosynthesis of type I collagen and the overexpression of the proteoglycan syndecan-1, re-enforcing their ability to form a tumor-permissive environment. It becomes thus evident that counteracting senescent cells in the stroma could represent an additional molecular target along with the efficient elimination of neoplastic cells in generalized anticancer treatment regimes.

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P5

Genome editing of an essential tRNA^{Gly} ligand impairs T-box riboswitch-mediated sensing of amino acids and triggers differential global gene expression

Adamantia Kouvela[1#], Nikoleta Giarimoglou[1#], Alexandros Maniatis[1], Jinwei Zhang[2], Vassiliki Stamatopoulou[1*], Constantinos Stathopoulos[1*]
[1]Department of Biochemistry, School of Medicine, University of Patras, 26504 Patras, Greece
[2]Laboratory of Molecular Biology, National Institute of Diabetes and Digestive and Kidney Diseases, 50 South Drive, Bethesda, MD 20892, USA
**cstath@med.upatras.gr; v.stam@upatras.gr*
 # equal contribution

Staphylococcus aureus, a prominent and highly antibiotic-resistant human pathogen, possesses a previously characterized unique T-box riboswitch which controls transcription of *glycyl*-tRNA synthetase (GlyRS) and affects both translation and cell wall formation[1]. This *glyS* T-box contains an appended and species-specific stem (stem Sa) in the antiterminator domain, which ensures specificity against the encoded 5 tRNA^{Gly} isoacceptors and serves as a hotspot for binding of mainstream antibiotics that inhibit protein synthesis[2,3]. The elusive, so far, role of each tRNA^{Gly} isoacceptor in the synchronization of both pathways was examined for the first time, using a special CRISPR/Cas9 genome editing tool designed for *S. aureus*. The P1 tRNA^{Gly} which is the major and more specific ligand of the *glyS* T-box was successfully ablated in the appropriate *S. aureus* RN4220 strain. Subsequent NGS analysis of the edited strain revealed alterations in the expression of all the remaining components that are affected by the T-box riboswitch (tRNA isoacceptors and GlyRS) as well as in the expression of other genes. Although the profile alterations of wild-type and edited strains were not excessive, subsequent gene enrichment ontology analysis showed that central biological processes such as the phosphate pentose pathway, the purine metabolism pathways and the homeostasis of metal ions was affected. In addition, alterations in expression of genes involved in cell wall organization, downregulation of toxins and host defense proteins were also observed. Finally, we observed alteration in the expression of genes encoding for the ABC transporter-ATP binding protein and FMN biosynthetic pathway which is known to be extensively regulated by riboswitches. Our results show for the first time that the absence of tRNAs that have been previously considered indispensable for the cells, is not lethal but strongly affects bacterial homeostasis possibly through novel RNA-mediated regulatory circuits of different types of riboswitches.

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P6

Exploring the effects of pharmacological modulation of the circadian clock on the oncogenic properties of cancer cell lines

Danae Farmakis[1,2], Dimitrios J. Stravopodis[2], Anastasia Prombona[1*]
[1]Institute of Biosciences and Applications, NCSR "Demokritos", Aghia Paraskevi, Greece
[2]Section of Cell Biology and Biophysics, Department of Biology, National and Kapodistrian University of Athens (NKUA), Greece
**e-mail: prombona@bio.demokritos.gr*

The circadian clock is a highly conserved autonomous mechanism which confers a 24-hour rhythmic function on a plethora of physiological processes of many organisms and has evolved as an adaptation to the Earth's rotation and the day/night transitions. 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 with cancer development and progression. Moreover, clock gene or protein expression is dramatically attenuated particularly in higher stage or aggressive tumors. RNA-sequencing and immunohistochemistry data from various cancer tissue samples reveal in most cases low expression of core circadian genes and proteins compared to healthy adjacent tissues, associating the deregulation of the circadian clock with cancer progression and poor 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. The aim of the current study is to explore whether clock modulators can alter the oncogenic characteristics of selected cancer cell lines and to investigate whether they can be used to improve or restore circadian oscillation and function.

P7

Histone Epigenetic Changes Induced by the Histone Deacetylase Inhibitor Sodium Butyrate in Human Leukemic Cells as Compared to Normal Peripheral blood Lymphocytes

Anastasia-Georgia Dedemadi[1], Thomae G. Sourlingas[1]*
[1]Nuclear Proteins and Chromatin Function Lab, Institute of Biosciences and Applications, National Centre for Scientific Research "Demokritos"

Histone deacetylase inhibitors (HDACIs) can be used to investigate histone epigenetic regulation of chromatin function. Moreover, they have been used alone or in combination with other agents in anticancer therapeutic regimens. For these reasons, we wished to analyze effects of the HDACI, sodium butyrate (SB), on histone H3 posttranslational modifications (H3K9/14ac, H3K4me3, H3K9me2) and on histone H1 subtype/variant (H1.0, H1.3, H1.5) levels in three leukemic cell lines (K562, NB4, Molt4) as compared to normal lymphocytes. With SB there were statistically significant increases in the levels of H3 acetylation and H3 trimetylation, but no changes in the levels of H3 dimethylation. The former are associated with active euchromatin, while the latter with inactive heterochromatin. Of significance was the finding that while the levels of H1.0 increase with butyrate, we also observed a concomitant statistically significant decrease in H1.3 expression levels. H1.0 has been well-studied. However, HDACI-induced changes in the expression levels of H1.3 have not been previously reported. Moreover, these findings are of major importance since both H1.0 and H1.3 have been associated with chromatin regulation, in contrast with H1.5, which is associated with inactive heterochromatin and was not found to change with SB. Significant is also the fact that the increase in H1.0 levels with the concomitant decrease in H1.3 levels is effected by SB only in the leukemic cells and not in normal lymphocytes. These results parallel butyrate-induced mortality rates. No changes in cell numbers were found in lymphocytes, while statistically significant decreases in cell numbers were found in the NB4 and Molt4 cells lines, both of which showed the greatest epigenetic differences in the presence of SB. One area of investigation that these results lead to is that certain histone epigenetic factors can be used as biomarkers to estimate the targeted efficiency/effectiveness of HDACIs in anticancer therapeutic regimens.

Keywords: histones; epigenetics; sodium butyrate; leukemia cells; normal lymphocytes

P8

Time-of-day interacting partners of poly(A)-specific ribonuclease in mouse liver

Rafailia A.A. Beta[1], Zoi V. Arsenopoulou[1], Martina Samiotaki[2], Dimitrios Dalkidis[1], Dimitris Kletsas[3], George Panayotou[2], Nikolaos A.A. Balatsos[1]

[1] Department of Biochemistry and Biotechnology, University of Thessaly, Biopolis 415 00, Larissa, Greece
[2] Biomedical Sciences Research Center "Alexander Fleming", Vari 16672, Greece
[3] Laboratory of Cell Proliferation & Ageing, Institute of Biosciences & Applications, National Centre for Scientific Research 'Demokritos', Athens, Greece
e-mail: balatsos@bio.uth.gr

Circadian rhythms are inert time-keeping mechanisms helping organisms respond to periodic stimuli by regulating the rhythmicity of gene expression and mRNA lifespan. The poly(A) tail of RNAs is critical for mRNA stability, thus directly determines mRNA lifespan. The length of the tail is variable throughout the day as a result of poly(A) elongating and shortening enzymes. Nevertheless, the removal of the poly(A) tail is the first step that triggers the degradation of mRNA and is performed by enzymes called deadenylases. Despite that several deadenylases have been identified, their rhythmic expression and roles in circadian gene expression are poorly investigated. Therefore, it remains unclear whether specific deadenylases are rhythmically expressed and how circadian transcripts are degraded. Herein, we investigate the interacting network of PARN in mouse liver over the timecourse of 24-h. To this end, we immunoprecipitated PARN in C57BL/6J liver extracts throughout the 24h-day and performed mass spectrometry analysis of the eluted proteins. We find that PARN has different interacting partners under normal light/dark conditions relative to constant darkness conditions. Apart from PARN's major role in RNA metabolism, as is also highlighted in our data, its interactive partners involve PARN to distinct biological processes, such as mitochondrion and ER network organization, translation and transport, among others. Taken together, these results pave the way to elucidate interacting partners of PARN that regulate activity and dictate target specificity in the circadian clock.

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P9

Identification of compounds with anti-aggregation properties with emphasis on proteolytic mechanisms

Yiorgos Paikopoulos[1,2], Eleni Panagiotidou[1,2], Anna Gioran[1], Virginia D. Dimaki[3], Vassiliki Iconomidou[4], Fotini N. Lamari[3], Niki Chondrogiani[1*]
[1]*National Hellenic Research Foundation, Athens, Greece*
[2]*Department of Biochemistry and Biotechnology, University of Thessaly, Larissa, Greece*
[3]*Department of Pharmacy, University of Patras, Patras, Greece*
[4]Department of Biology, National and Kapodistrian University of Athens, Athens, Greece
* corresponding author

Protein homeostasis (proteostasis) refers to the molecular mechanisms that are responsible for the maintenance of the cellular protein network. Proteostatic mechanisms tend to decline with age and this often leads to accumulation of toxic protein aggregates. The Aβ peptide that has been causally related to Alzheimer's disease (AD) onset and progression represents one of these proteins aggregation-prone proteins. Plant secondary metabolites have been shown to be beneficial for proteostasis maintenance and/or restoration. Here, we have searched for natural products with anti-aggregating properties from the Greek flora, using mainly *C. elegans* AD models for screening. 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 compounds that are responsible for these properties. Since the autophagy-lysosome system (ALS) is a proteolytic mechanism that has been implicated in the elimination of protein aggregates, we further investigated the potential autophagy-activating properties of the isolated natural products. Our preliminary results implicate autophagy in the protective action of the isolated bioactive compounds.

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P10

Siramesine, a non-opioid sigma receptor agonist as potential agent for the development of novel targeted treatments for pancreatic cancer

E. Sereti[1], F. Koutsougianni[2], C. Tsimplouli[2], M. Pešić[3], J. Dinić[3], A. Divac Rankov[4], E. Armutak[5], A. Uvez[5], K. Papaparaskeva[6], C. Dervenis[7], A. Lambrianidou[2], N. Sakellaridis[2], K. Dimas[2]

[1] *Lund University, Department of Translational Medicine, Malmö, Sweden*
[2] *University of Thessaly, Department of Pharmacology, Larisa, Greece*
[3] *University of Belgrade, Institute for Biological Research "Siniša Stanković, Serbia*
[4] *University of Belgrade, Institute of Mol Genetics and Genetic Engineering, Serbia*
[5] *Istanbul University, Histology, and Embryology, Istanbul, Turkey*
[6] *Konstantopouleio General Hospital, Histopathology Department, Athens, Greece*
[7] *Hepatobiliary and Pancreatic Surgery Clinic, Metropolitan Hospital, Athens, Greece*

This study aimed to investigate the anticancer activity of siramesine (SRM) on human pancreatic ductal adenocarcinoma (PDAC). In this context, we studied, siramesine's in vitro and in vivo efficacy either as monotherapy or in combination with gemcitabine (GEM) in a xenograft model from a patient with PDAC cancer (PDX). Initially, we studied the levels of σ receptors by western blot analysis in two patient derived ex vivo pancreatic cancer cell populations, isolated and developed in our laboratory. In vitro evaluation of SRM against these cells followed. Finally, the in vivo efficacy of siramesine was tested in the corresponding PDX. Toxicity evaluation of SRM was performed in zebrafish including evaluation of the effects in embryo development and overall mortality and in NOD/SCID mice and. In vitro studies showed that SRM could kill primary pancreatic tumor cells via both autophagic death and apoptosis induction. Subsequent studies of SRM against a PDAC PDX developed in our laboratory showed a good anticancer activity. A major disadvantage of SRM appears to be its dose-dependent toxicity. Of note, our experimental data show that SRM can improve the efficacy of GEM when administered in combination in this PDX model. Finally the study of the mechanism of action of SRM in tumors, in good agreement with the in vitro data, showed the induction of both apoptosis and autophagic cell death in the PDAC xenografts. Support: RESEARCH–CREATE–INNOVATE, grant T1EDK-01612 and T1EDK-01833.

P11

Regulatory interplay by ERK1/2 and CK1δ signaling controls HIF-1α association with microtubules

Christina Arseni[1], Eleni Datsi[1], Martina Samiotaki[2], George Panagiotou[2], George Simos[1], Ilias Mylonis[1]*

[1]Laboratory of Biochemistry, Faculty of Medicine, University of Thessaly, Larissa, Greece
[2]Institute for Bio-innovation, BSRC "Alexander Fleming", Vari, 16672, Greece.
**e-mail: mylonis@med.uth.gr*

Hypoxia inducible factor 1 (HIF-1), a heterodimeric transcription factor activates the transcription of genes that are involved in the cellular response to hypoxia and are crucial for promoting tumor development. Expression of HIF-1α subunit is controlled by oxygen levels but its regulation also depends on post-translational events such as phosphorylation. Our previous work has shown that phosphorylation of HIF-1α by ERKs at Ser641/643 promotes HIF-1α activation by increasing its nuclear accumulation and its association with NPM1 and chromatin components[1]. Inhibition of this phosphorylation leads to HIF-1α nuclear export and its association with mortalin in an anti-apoptotic complex on the surface of mitochondria[2]. On the other hand, CK1δ phosphorylates HIF-1α at Ser247 and impairs the formation of a functional HIF-1α/ARNT heterodimer[3]. To better understand the possible interplay between these two antagonistic modifications and their functional significance for cancer cell adaptation to hypoxia, we constructed double HIF-1α mutants by combining mutations that either mimic (S247D, S641E) or impair (S247A, S641,643A) phosphorylation at the respective modification sites. Among the four different GFP-HIF-1α double-site mutant constructs, SDSA (S247D/S641/3A) was the only form that was localized outside the nucleus and exhibited no transcriptional activity. Analysis by mass spectrometry of proteins co-immunoprecipitated with GFP-HIF-1α SDSA from HeLa cell extracts, revealed SDSA association with mitochondrial proteins (as expected by the lack of its ERK1/2 phosphorylation) and, unexpectedly, with components of microtubular structures. In-vitro binding and immunoprecipitation assays using HIF-1α fragment 1-347 carrying mutations at position 247 (S247D and S247A) validated the phosphorylation-dependent association of HIF-1α with tubulin. Immunofluorescence microscopy experiments are currently under process to corroborate the localization of CK1δ-modified HIF-1α with microtubules. Overall, our results suggest that, in the absence of ERK activation, CK1δ regulates the association of HIF-1α with the cytoskeleton.

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P12

Design of a Custom RT-qPCR Array for Assignment of Resistance to Nervous Necrosis Virus in European Sea Bass *(Dicentrarchus labrax)*

Dimitra K. Toubanaki[1], Odysseas P. Tzortzatos[1], Antonia Efstathiou[1], Leonidas Papaharisis[2], Vasileios Bakopoulos[3], Evdokia Karagouni[1]
[1]*Hellenic Pasteur Institute, Department of Microbiology, Vas. Sofias 127, 11521, Athens, Greece;*
[2]*Nireus Aquaculture S.A., 1st klm. Koropiou-Varis Avenue, 19400 Koropi, Greece;*
[3]*University of the Aegean, Department of Marine Sciences, 81100 Mytilene, Greece.*
**e-mail: ekaragouni@pasteur.gr mailto:author@yahoo.com*

Widespread aquaculture losses attributed to pathogen infections frequently occur in fish farms, especially in the Mediterranean area. The European sea bass (*D. labrax*) a teleost fish extensively farmed with high economic importance, is highly susceptible to diseases of viral aetiology, especially viral nervous necrosis (VNN). Recent studies correlate the host genetic background to resistance in nervous necrosis virus (NNV), the disease causing pathogen. With that scenario in mind, the gene expression profiles of one resistant and one susceptible *D. labrax* family were evaluated following experimental infection with VNN via RNA-Seq analysis. **Aim of the present study was the construction of a custom qPCR array to identify genes related to NNV resistance in *D. labrax***, based on the differentially expresses genes (DEGs) recognized by RNA-Seq data analysis. Sea-bass from the two families were challenged with NNV and sampled at 3 hours, 48 hours and 14 days post-challenge, followed by re-infection and sample collection after 7 days. The resistant family showed substantially higher numbers of both up-regulated and down-regulated transcripts than the susceptible family in all time-points prior re-infection. In order to characterize genes that are associated with NNV resistance, the gene sets which were up- or down regulated in each family were compared with Venn diagrams. A total of 41 genes were up-regulated specifically in infected resistant family whereas 24 genes were up-regulated in infected sensitive family. Based on their function, 45 genes were selected to build a custom qPCR array. The genes were uniquely up-regulated in each family (resistant and sensitive) or commonly up-regulated in infected and non-infected fish for each family and their functions were related to immune response, metabolism and cytoskeletal components. It is anticipated that the developed tool will be applied to assess European sea bass resistance to NNV infection on field.

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P13

Exploring the inhibitory effect of a kaurene diterpene on the aggregation of Amyloid beta peptide

George Tzanetoulakos[1], Georgia I. Nasi[1], Panagiotis M. Spatharas[1], Aikaterini Evangelia I. Rizou[1], Virginia D. Dimaki[2], Fotini N. Lamari[2], Vassiliki A. Iconomidou[1]*
[1]Section of Cell Biology and Biophysics, Department of Biology, School of Science, National and Kapodistrian University of Athens, Panepistimiopolis, Athens 157 01, Greece,
[2]Laboratory of Pharmacognosy and Chemistry of Natural Products, Department of Pharmacy, University of Patras, 26504 Patras

Nowadays, fifty million people are affected by the devastating impact of the most common neurodegenerative disease, called Alzheimer's disease (AD). It is widely accepted that one of the pathological hallmarks of AD is senile plaques which mainly consist of amyloid fibrils. The major component of these extracellular deposits is amyloid beta (Aβ) peptide which derive from the degradation of amyloid precursor protein (APP) by β- and γ-secretase. Regarding the therapeutic approach for AD, only two drug categories exist, the cholinesterase inhibitors and the N-methyl D-aspartate (NMDA) antagonists. In spite of the increasing number of studies, the available drugs are aiming to treat the symptoms and not to prevent or cure AD. Recently, natural extracts have been evaluated for their neuroprotective effects. Taking into consideration that natural products seem to have therapeutic potential, we attempted to elucidate the role of a natural product, siderol, in the formation of Aβ amyloid fibrils. Specifically, $A\beta_{42}$ peptide was chemically synthesized and the ability of siderol to inhibit its aggregation was studied *in vitro*, utilizing Congo Red staining and polarized light microscopy, negative staining and Transmission Electron Microscopy, and Thioflavin T (ThT) kinetic assays. Overall, the findings of our experiments suggest that the natural product siderol is efficient at delaying the amyloid fibrils formation of Aβ peptide. Even though inhibition of Aβ peptide aggregation was not clearly observed, further investigation could lead to the exploitation of siderol or analogues of it as a drug candidate.

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P14

Analysis of the transcriptome in modelled osteoporosis reveals new potent regulators of bone remodelling

Elisavet Ioannidou[1], Vagelis Rinotas[1], Marios Itsios[1,3], Lenka Plestilova[2], Astrid Jüngel[2], Trias Thireou[3], Douni Eleni[1,3]*

[1]Institute for Bioinnovation, Biomedical Sciences Research Center "Alexander Fleming", Vari, Greece
[2]Center of Experimental Rheumatology, University Hospital Zürich, Zurich, Switzerland
[3]Laboratory of Genetics, Department of Biotechnology, Agricultural University of Athens, Athens, Greece

Osteoporosis is a multifactorial disease characterized by bone loss, bone fragility and increased bone fracture risk. It is often referred as a silent epidemic since it is often underdiagnosed 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). To identify differentially expressed (DE) genes with potential clinical value in osteoporosis, we performed RNA-Seq for mRNAs and miRNAs in flushed femurs of TgRANKL mice. 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 wild type (WT). Enrichment analysis of the upregulated genes revealed that they were related to protein degradation, proteolytic enzymes, transport, response to cytokines, cell adhesion, apoptosis, and bone remodelling, while downregulated genes were mainly related to metabolism, transport, cytoskeleton organization, muscle structure, oxidative phosphorylation, a variety of signalling pathways. We confirmed with qPCR, 10 upregulated genes, and 3 downregulated. 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, 3 upregulated and 3 downregulated miRNAs. 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.

P15

Fibromine: a tool for centralized exploration and integration of pulmonary fibrosis omics data

Dionysios Fanidis[1], Panagiotis Moulos[2]*, Vassilis Aidinis[1]*
[1]*Institute for Bioinnovation, Biomedical Sciences Research Center Alexander Fleming, 16672 Athens, Greece*
[2]*Institute for Fundamental Biomedical Research, Biomedical Sciences Research Center Alexander Fleming, 16672 Athens, Greece*

Idiopathic pulmonary fibrosis (IPF) is an incurable disease of the lungs with limited treatment options available. A great number of omics profiling studies have contributed crucially to the discovery of molecular mechanisms implicated in this heterogeneous pathology. Despite this huge volume of publicly available data, there have been only sparse attempts aiming to their full utilization via their comparative analysis and/or integration. In this context, we here present Fibromine, a manually curated database and mining server dedicated to IPF omics data. Fibromine comprises of a great collection of carefully selected, consistently re-analyzed bulk transcriptomic and proteomic datasets extending over a wide spectrum of experimental designs in both patients and animal models. Our database can be accessed via the homonym R Shiny application (http://www.fibromine.com/Fibromine) offering various dynamic data exploration and on-the-fly integration functionalities. These include, among others, intra- and inter- species datasets integration, creation of IPF-specific protein-protein interaction and gene co-expression networks, as well as a list of potentially interacting differentially expressed mRNAs-miRNAs. In parallel, we introduce a novel data-driven transcriptomic datasets benchmarking system, leading to the accreditation of the most "homogeneous" datasets, in an attempt to describe data variability in a non-invasive and scalable fashion. Moreover, to exploit the cutting-edge single cell resolution studies emerging, Fibromine supports the visualization and/or exploration of several scRNA-seq datasets as a first step towards their integration with the legacy of bulk omics data. Conclusively, Fibromine constitutes a centralized hub for real-time investigation and integration of many otherwise scattered publicly available IPF omics datasets, requiring no computational expertise from its users. Hopefully, this endeavor will assist to the formation of new hypotheses regarding fibrosis, to the quick and painless validation of new experimental findings and to the discovery of novel disease targets.

P16

From two to many: McIdas controls centriole numbers in cells

Marina Arbi[1], Margarita Skamnelou[1], Spyridoula Bournaka[1], Sihem Zitouni[2], Ozge Karayel[3], Catherine G. Vasilopoulou[3], Aikaterini Tsika[4], Georgios Spyroulias[4], Matthias Mann[3,5], Monica Bettencourt-Dias[2], Stavros Taraviras[6] and Zoi Lygerou[1*]

[1]Department of General Biology, School of Medicine, University of Patras, Greece
[2]Instituto Gulbenkian de Ciência, Oeiras, Portugal
[3]Department of Proteomics and Signal Transduction, Max Planck Institute of Biochemistry, Martinsried, Germany
[4]Department of Pharmacy, University of Patras, Greece
[5]NNF Center for Protein Research, Copenhagen, Denmark
[6]Department of Physiology, School of Medicine, University of Patras, Greece

The number of centrioles in dividing cells is tightly controlled to ensure bipolar spindle assembly. Aberrations in centriole numbers lead to genomic instability and cancer[1]. The tight control of the expression levels and the interactions of PLK4, STIL and SAS6, which initiate centriole duplication, have been proposed as predominant mechanisms for the regulation of centriole numbers, to avoid reduplication in the same cell cycle. However, our understanding of the fundamental principles that govern this process is still poor. Here, we characterized McIdas as a novel protein that contributes to a normal centriole cycle. McIdas along with Geminin and GemC1 constitute the Geminin superfamily with significant roles both in cell cycle[2,3] and in centriole amplification during the differentiation of multiciliated cells[4-8].

McIdas depletion and over-expression experiments show that regulation of McIdas expression is important for the maintenance of correct centriole numbers in cancer and normal cycling and S-phase arrested cells. McIdas depletion in cells reduces centriole numbers, whereas its ectopic expression induces centriole amplification. Super-resolution microscopy was combined with mutant analysis to assess McIdas mode of function. Further analysis shows that McIdas affects the core centriole duplication machinery by interacting with the kinase PLK4. Consistently, McIdas depletion inhibits PLK4-induced centriole biogenesis. Post-translational modifications of McIdas protein, including its phosphorylation by PLK4, highlight its importance in centriole number control. PLK4-specific phosphorylation sites on McIdas protein were identified through mass-spectrometry and their significance in centriole numbers was analyzed.

The above data suggest that McIdas is important for the control of centriole numbers in cycling cells. McIdas and GemC1 are also implicated in centriole amplification in multiciliated cells. We propose that Geminin family proteins control these two different centriole biogenesis pathways in cells and may contribute to the coordination of the chromosome and centrosome cycles, safeguarding cell homeostasis and genome integrity.

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P17

Inhibition studies of protein-derived peptide-analogues on Aβ peptide aggregation

Anna G. Oikonomidou[1], Georgia I. Nasi[1], Panagiotis M. Spatharas[1], Athina Lykoura[2], Aimilia D. Sklirou[1], Ioannis P. Trougakos[1], Vassiliki Magafa[2], Vassiliki A. Iconomidou[1*]
[1]Section of Cell Biology and Biophysics, Department of Biology, School of Science, National and Kapodistrian University of Athens, Panepistimiopolis, Athens 157 01, Greece
[2]Laboratory of Pharmacognosy and Chemistry of Natural Products, Department of Pharmacy, University of Patras, 26504 Patras
**email: veconom@biol.uoa.gr*

Alzheimer's Disease is the leading cause of dementia accounting for approximately 60-80% of all dementia cases and as such a plethora of therapeutics have been and are still currently being tested. One of its main neuropathological hallmarks is the formation of extracellular proteinaceous deposits, known as amyloid plaques, which consist primarily of amyloid beta (Aβ) peptide. However, several other components have been detected in these deposits including the so-called co-localized proteins. One of the therapeutic approaches for Alzheimer's Disease is based on the inhibition of Aβ peptide aggregation. A variety of potential inhibitors have been tested, with the majority of them being peptides. The co-deposited proteins found in amyloid plaques can be a source of peptide-inhibitors and in Alzheimer's disease one such example is the lysosomal endonuclease cathepsin D. In this work, AMYLPRED2, a consensus algorithm which predicts protein regions that exhibit increased aggregation propensity, was used to locate "aggregation-prone" segments in the mature cathepsin D sequence. A select few of the predicted regions were chemically synthesized and then co-incubated with the Aβ peptide *in vitro*. With the aid of molecular biophysical techniques, we were able to observe and determine whether the peptide-analogues had any inhibitory effects on Aβ aggregation. The methods used included Transmission Electron Microscopy after negative staining, Congo Red Birefringence and Thioflavin T (ThT) fluorescence assays. Our results revealed that some of the peptide-analogues appear to inhibit and/or delay the aggregation of the Aβ peptide.

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P18

Tryptophan: A key residue for Arkadia-2C interaction with Ubch5b enzyme. An essential component for in vitro ubiquitination

Konstantinos D. Marousis[1], Maria D. Politi[1], Anastasia Seliami[1], Revekka D. Karavela[1], Maria Birkou[1], Vasso Episkopou[2], Georgios A. Spyroulias[1]
[1]Department of Pharmacy, University of Patras, Patras, Greece, [2]Imperial College, London, United Kingdom
**email: g.a.spyroulias@upatras.gr*

The ubiquitin-proteasome system is an essential tool for the regulation of proteins level in the cells. Specifically, ubiquitin (Ub) marks the targeted protein for degradation by the 26S proteasome. The attachment of Ub requires the concerned actions of three enzymes, the E1 Ub-activating enzymes, the E2 conjugating enzymes and the E3 Ub ligases. The E3 enzymes are the key components of the process as they are responsible for substrate recognition and poly-ubiquitination[1]. Arkadia and Arkadia-2C proteins act as E3 Ub ligases via their C-terminal RING domains. Arkadia is a positive regulator of the TGF-β pathway[2] whilst Arkadia-2C is implicated, as a positive regulator, in the BMP pathway[3]. Kelly et all [3]demonstrated that Arkadia-2C enhanced BMP signaling is involved in axon growth. The two proteins are 85% identical in the sequence of their RING domains.
Nuclear Magnetic Resonance (NMR) driven interaction studies of Arkadia, with the E2 enzyme UbcH5b revealed a major role of Arkadia tryptophan (Trp) in UbcH5b recognition and binding[4]. Trp is considered as one of the key residues for E2 recognition and binding and is in the α-helix of RING domain in many proteins with proven E3 Ub ligase activity. Replacement of this Trp by Ala in RING domains of proteins such as EL5, c-Cbl, and Kaposi's sarcoma-associated Herpesvirus K3 variant leads to a reduced affinity for E2 enzyme and a complete loss of E3 activity. Arkadia mutants, where Trp is replaced by Ala or Arg, were studied, demonstrating that the effect of Trp replacement depends on the nature of the residue at this position[5]. Interestingly, study of the effect of Trp to Ala or Arg mutations on Arkadia-2C RING E3 ubiquitin showed different profile of the interaction with the E2 enzyme, as well as in the *in vitro* ubiquitination assays.

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P19

Cross talk between Wnt-1-induced signaling protein 1 (WISP-1) and macrophage migration inhibitory factor (MIF) in primary human lung fibroblasts

M-E. A. Christopoulou[1,2]*, D. Stolz[1], E. Papakonstantinou[1], A. J. Aletras[2]
[1]*Department of Pneumology, Medical Center - University of Freiburg, Faculty of Medicine - University of Freiburg, Freiburg, Germany;*
[2]*Biochemistry, Biochemical Analysis & Matrix Pathobiology Res. Group, Laboratory of Biochemistry, Department of Chemistry, University of Patras, Greece*

WISP-1, a member of the CCN matricellular proteins family, is increased in the epithelium of patients with lung diseases, indicating a role of this protein in the pathophysiology these diseases. MIF is a proinflammatory cytokine, present in lung, able to induce the expression of other cytokines, such as IL-6 and prostaglandins. The aim of the present study was to investigate if there is a cross talk between WISP-1 and MIF in the lung and the intracellular mechanisms involved. Primary human lung fibroblasts (LFs) were treated with WISP-1 (1-100 ng/ml) and the expression of MIF and IL-6, at the level of mRNA and protein, was assessed by real time PCR, and ELISA and western blot, respectively. The effect of Wisp-1 on EGFR activation was studied by immunoprecipitation, using specific antibodies against EGFR and phosphorylated Tyrosin. WISP-1 stimulated significantly the expression of MIF and IL-6 in LFs in a concentration- and time-dependent manner. WISP-1-induced MIF expression was mediated by the αvβ5 integrin and dermatan sulphate proteoglycans, as well as by the PKC, MAP kinases, PI3-K/Akt and NF-κB signalling pathways, and was attenuated in the presence of PP2, an inhibitor of src kinases. WISP-1 significantly increased EGFR phosphorylation, even in the presence of EGFR tyrosine kinase activity inhibitor, AG1478, which was completely suppressed in the presence of src kinases inhibitor, PP2. WISP-1-induced IL-6 expression was suppressed in the presence of ISO-1, an inhibitor of MIF tautomerase activity, indicating that MIF is involved in this effect. In addition, WISP-1 enhanced the expression of COX-2 and the production of prostaglandin E2 (PGE_2) by LFs, while treatment with ISO-1 resulted in the abrogation of this effect, indicating the involvement of MIF. PGE_2 was also able to enhance the production of IL-6 from LFs, an effect that was mediated through trans-activation of EGFR by src kinases. The implication of WISP-1 in the pathophysiology of lung diseases may be mediated by the increased expression of MIF and its stimulatory effect on the production of the known pathogenetic agents, IL-6 and PGE_2 from LFs. The WISP-1-induced MIF expression is mediated by specific signaling pathways which may be proven to be excellent candidates as novel targets to control inflammation in chronic lung diseases.

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P20

Greek Medicinal Plants for Photoprotection

Barbara Mavroidi[1*], Efstathia Thoma[2], Aikaterini Argyropoulou[2], Alexios-Leandros Skaltsounis[3], Maria Pelecanou[1]

[1] Institute of Biosciences & Applications, National Centre for Scientific Research "Demokritos", Athens, Greece,

[2] PharmaGnose S.A., 57th km Athens-Lamia National Road, Oinofyta, 32011, Greece &

[3] Division of Pharmacognosy and Chemistry of Natural Products, Faculty of Pharmacy, University of Athens, Panepistimiopolis, Athens, Greece

**e-mail: bmavroidi@bio.demokritos.gr*

One of the most damaging actions on human skin comes from solar radiation, especially from its ultraviolet (UV) component, leading to histologic damage such as hyperpigmentation, photoaging, photosensitivity and skin cancers [1-2]. Medicinal plants and their extracts possess a vast and complex arsenal of phytochemicals with well reported photoprotective effects against UV-induced damages [3]. As Greece is considered one of the global ecological "hot spots" with rich heritage in Medicinal and Aromatic Plants (MAPs) hosting the most diverse flora in the Mediterranean [4], in this project, a variety of extracts from aromatic and medicinal plants collected from the wild or cultivated in Greece, are biologically evaluated for UV-protection and antiaging activity against a panel of healthy human epidermal cell lines. In a first screening in cell-free assays, the extracts displaying strong UV absorption - a requirement for antioxidant and anti-hyperpigmentating and photoprotective action - are selected. Then, *in vitro* biological evaluation of their cytotoxicity, along with antioxidant and antiaging mechanistic investigations follows in healthy cells before and after UV irradiation. Results so far indicate that certain extracts from mountain tea and olive leaves have suitable properties to be used as cosmeceuticals, as they show low to non-existent toxicity and strong antioxidant activity. The assessment of the protective or therapeutic effects of the extracts against UV-induced cell damage is currently in progress with irradiation experiments covering both the UVA and UVB range, in order to evaluate the potential of the selected plants to act as sun photoprotective agents for cosmetic and medical purposes.

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P21

SH4 domain: a novel regulatory mechanism for Lck activation and signaling transduction initiation

Nikolaos Koutras*, Eleftheria Kissa and Konstantina Nika
Department of Biochemistry, School of Medicine, University of Patras, Patras, Greece

Lck is a member of the Src family of tyrosine kinases (SFKs), indispensable for signal transduction responses governing cell adhesion, motility, proliferation, and differentiation. This is achieved via the direct phosphorylation of conserved Tyrosine-containing motifs within the cytoplasmic tail of the T cell receptor (TCR). The expression of Lck is restricted to T lymphocytes where it is predominantly anchored in the inner leaflet of the plasma membrane, as a result of lipid modifications on the N-terminal SH4 domain.
Previous studies have indicated that there is a substantial pool of constitutively active Lck molecules within resting T cells, the existence of which is necessary and sufficient for TCR signaling. However, this pool of pre-activated Lck is allowed to act on its substrate only after TCR ligation. A mechanism explaining this observation, could be the finely orchestrated compartmentalization between Lck and its substrate in the plane of the plasma membrane.
The focus of this study is to investigate the impact of altering Lck's subcellular localization, on its activity and its ability to induce TCR signaling. Lck SH4-deletion mutants or chimeric Lck molecules in which the SH4 domain was substituted by the corresponding regions of other SFK members, were expressed in an SFK-deficient T cell line.
Our initial observations show that, following overexpression, Lck is capable of triggering TCR signaling pathways in the absence of TCR ligation. Further experiments revealed that Lck has the ability to achieve its active form and subsequently its functionality, only after anchoring to the plasma membrane. Finally, we showed that different subcellular localization of the chimeric SH4-Lck molecules, led to slight alterations in the tuning of the signaling responses, albeit the comparable levels of Lck activity among all molecules.
Our findings will contribute to understanding how molecular confinement within dedicated cellular compartments impacts the fine-tuning of enzyme-substrate interactions.

P22

The effect of high temperature on the intracellular sucrose accumulation of the cyanobacterial strains Synechococcus elongatus PCC 7942 and Synechocystis sp. PCC 6714

Panayiotis-Ilias Broussos[1], Elisavet Amanatidou[2], Kostas Stamatakis[1,*].
[1] *Biophysics and Biotechnology of Membranes Laboratory Institute of Biosciences and Applications, NCSR "Demokritos",Athens, Greece*
[2] *Department of Chemical Engineering, University of Western Macedonia, Koila, Kozani 50100, Greece*

Sucrose is one of the most important feedstocks for food industry and is widely used as a carbohydrate substrate for the production of clean fuels. A common approach towards its production is the use of photosynthetic organisms. Among them, cyanobacteria are the most suitable for the capture of excessive amounts of CO_2 and can be grown in extreme or special environmental conditions (eg temperature, pressure, salt content, pH, chemical composition), for the production of chemical compounds and energy. In the present study, we use two standard laboratory organisms, the unicellular freshwater cyanobacteria ***Synechococcus elongatus*** PCC7942 (S7942) and ***Synechocystis sp.*** PCC6714 (S6714), which, when exposed to high salinity, synthesize sucrose as their main compatible osmolyte. We examined the optimal growth temperature for the accumulation of sucrose in those two organisms, with respect to their photosynthetic capacity (in terms of Chlorophyll a/ml culture), in salted BG-11 medium (0,4M NaCl added). The sucrose production was favoured by high temperature (above 31°C standard growth temperature) and was maximized at 35°C in both organisms. In particular, the intracellular sucrose content per chlorophyll a was increased by 33% and by 52%, in the case of S7942 cells and of S6714 cells, respectively. The growth rate of S7942 remained constant and that of S6714 declined slightly during the first 7 days of the upshock at 35°C, then both increased and remained positive for the rest of the incubation periods. Overall, the quantities of the sucrose produced by S7942 and S6714 were enhanced significantly and may be sufficient as a viable alternative (a) to sucrose synthesis, and (b) to fuel formation such as H_2 or bioethanol, outside the finite freshwater reservoirs, while reducing the ambient CO_2.

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P23

The Tbx5 heart saga; from Development to Regeneration

Panagiota Siatra[1], Giannis Vatsellas[2], Athanasia Chatzianastasiou[3], Eleni Mouchtouri[1,4], Andreas Papapetropoulos[1,3], Dimitrios Thanos[1], Manolis Mavroidis[1], Dimitrios Beis[1] & Ioannis Kokkinopoulos[1*]

[1] *Center for Clinical Experimental Surgery and Translational Research, Developmental Biology Laboratories, Biomedical Research Foundation Academy of Athens, Greece*
[2] *Hellenic Genome Centre, Biomedical Research Foundation Academy of Athens, Greece*
[3] *Faculty of Pharmacy, National and Kapodistrian University of Athens, Greece*
[4] *Department of Cardiology, University of Ioannina, Greece*

Heart failure is the major cause of death in the industrialized countries, representing 30% of all global deaths. Injury to the adult mammalian cardiac muscle, often leads to a heart attack due to irreversible loss of cardiomyocytes, creating an unmet need for identifying idle cardiac regenerative mechanisms. Expression of the embryonic transcription factor Tbx5 is a hallmark of first heart field progenitors and paramount for differentiation towards a cardiomyocyte fate. Using a developmental approach to the adult heart injury mouse model *Tbx5^{Cre}/R26R$^{eYFP/eYFP}$* and by employing single-cell RNA-seq technology, we identify a Tbx5-expressing ventricular cardiomyocyte precursor cell population, in the injured adult mammalian heart. Our immunohistochemical analysis indicated the presence of YFP-expressing cells mostly around injury sites. The transcriptional profile of that precursor cell population is close to that of neonatal cardiomyocyte precursors. Taken together, our data reveal a cardiomyocyte precursor cell population, which is capable of dedifferentiating and potentially be involved in a cardiac regenerative program, providing us with an effective target for studies involving heart repair and regeneration. The role of Tbx5 in adult and postnatal mammalian cardiomyocyte regeneration needs to be further examined. Our ongoing efforts are focusing on identifying mechanisms that will allow for the expansion of the Tbx5 expression window, immediately after cardiac injury.

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P24

A Proteomic approach to study hepatitis C virus protein Core+1/Long biology

Vasileios Vrazas[1], Savvina Moustafa[2], Ioannis Karakasiliotis[3], Katerina R. Katsani[1*], Penelope Mavromara[1]

[1]*Laboratory of Biochemistry and Molecular Virology, Department of Molecular Biology and Genetics, Democritus University of Thrace, 68100 Alexandroupolis, Greece*

[2]*Department of Microbiology, Hellenic Pasteur Institute, 11521 Athens, Greece*

[3]*Laboratory of Biology, Department of Medicine, Democritus University of Thrace, 68100 Alexandroupolis, Greece*

Hepatitis C virus (HCV) is an enveloped positive-strand RNA virus of the Flaviviridae family, which causes liver inflammation and can lead to Hepatocellular Carcinoma (HC). Core+1/Long is an isoform encoded from an alternative +1 open reading frame (ORF) within the coding region of the virus capsid protein Core. Core+1/Long appears to be dispensable for the virus replication [1] and to promote cell proliferation [2]. Interestingly, anti-core+1 antibodies are detected in HCV-infected patients and presented an increased prevalence in HCC patients [3]. To acquire deeper understanding of the Core+1/Long biology we used comparative proteomics and studied the changes brought to the proteome of hepatoma Huh 7.5 cells by the stable expression of the Core+1/Long isoform. The GSEA analysis of the proteomics data gave indications that Core+1/Long may play an important role in the development of advanced liver diseases in patients infected by HCV.

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P25

The inability of senescent WJ-MSCs to regulate Primary Cilium formation is possibly mediated by caveolin-1 dependent autophagy dysregulation

Zozo Outskouni[1], Aikaterini Latsi[1], Andreas Goutas[1], Varvara Trachana[1*]
[1]Department of Biology, Faculty of Medicine, University of Thessaly, Larisa, Greece

The Primary Cilium (PC) is a non-motile microtubule based organelle that protrudes from the plasma membrane of nearly all cells and acts as a cellular antenna for various signals, while also participating in signal transduction. Autophagy and primary cilia appear to be interconnected since studies have shown that autophagy is involved in primary cilia formation and functional primary cilia are important for autophagy regulation. Moreover autophagy and caveolin-1 -a plasma membrane protein required for the generation of membrane invaginations called caveolae- are also closely linked, while both caveolin-1 and autophagy exhibit a regulatory role in senescence, a condition characterized by stable cell cycle arrest. Given the above we aimed at investigating the relationship between autophagy, primary cilia and caveolin- 1 during cellular senescence of Mesenchymal Stem Cells derived from the Wharton's Jelly (WJ-MSCs). WJ-MSCs are a valuable tool in the field of regenerative medicine due to their multipotency and lower immunogenicity compared to MSCs isolated from other sources. We performed immunofluorescence assay and western blot analysis for acetylated-a-tubulin (the basic ciliary axoneme protein) in early and late passage WJ-MSCs, under serum starvation and under caveolin-1 silencing. Our results showed that primary cilia formation is dysregulated in senescent WJ-MSCs, since the percentage of ciliated cells is elevated under normal conditions. Furthermore late passage cells failed to induce autophagy in response to serum starvation which coincides with their inability to form primary cilia under the same conditions. Caveolin-1 levels appeared elevated in senescent cells and silencing of caveolin-1 increases cilia formation and autophagy. Thus, we propose that caveolin-1 is involved in autophagy dysregulation which possibly contributes to the dysregulation of primary cilia formation and possibly senescence onset.

P26

Neuroprotective role of the orphan nuclear receptor Nr5a2

Ismini Rozani[1], Efstathia Tetringa[1], Katerina Dimitropoulou[1], Panagiotis K. Politis[1*]
[1]Biomedical Research Foundation of the Academy of Athens

Neurodegenerative diseases, brain injury, ischemia and inflammation are only some of the CNS-impairing diseases which severely affect millions of people worldwide, leading to death, disability and a plethora of comorbidities. Throughout the human life, neural tissue is exposed to a variety of injurious and toxic stimuli. The postmitotic status of neurons, as well as their limited regenerative capacity restricts their ability to overcome cellular injury and death. Common pathophysiological mechanisms such as oxidative stress, hypoxia and ischemia, neuroinflammation, excitotoxicity, toxicity due to protein aggregation and ER stress underline the majority of neurological and neurodegenerative diseases, culminating in neuronal death. Hence, the discovery of neuroprotective factors against multiple injuries has been at the forefront of biomedical research. In the present study we focus on the potential neuroprotective role of Nr5a2, an orphan nuclear receptor, with known pharmacological agonists and transcriptional activity. Nr5a2, also known as Lrh1, has been implicated in the embryogenesis of various visceral organs and in the regulation of metabolic processes, although its role in the CNS has not been extensively described. Here, we present evidence that Nr5a2 possesses a neuroprotective capacity in vitro, as shown on both SHSY-5Y cells and mouse embryonic primary neuronal cultures exposed to various insults. Nr5a2 overexpression increases cell survival after exposure of neuronal cells to H2O2, while it decreases activated caspase 3 expression at the protein level. Incubation of primary neurons with DLPC (1,2-dilauroyl-sn-glycero-3-phosphocholine), an Nr5a2 agonist, leads also to decreased expression of the activated caspase 3 protein. Nr5a2 is a druggable receptor that could become a promising neuroprotective target in various neurological disorders.

P27

Integrative analysis of cutaneous melanoma based on genomic, transcriptomic and dermoscopic data

Georgia Kontogianni[1,2], Georgia Piroti[1], Katerina Kypreou[3], Alexios Zaras[3], Erietta Christofidou[3], Amerini Dimitrakopoulou[3], Irene Stefanaki[3], Eleftherios Pilalis[4], Haralampos Karanikas[5,6], Athanasios Kallipolitis[7], Konstantinos Moutselos[7], Ilias Maglogiannis[7], Alexander Stratigos[3], Aristotelis Chatziioannou[2,4] and Olga Papadodima[1]

[1]Institute of Chemical Biology, National Hellenic Research Foundation, Athens, Greece
[2]Centre of Systems Biology, Biomedical Research Foundation of the Academy of Athens, Greece
[3]1st Department of Dermatology, Andreas Syggros Hospital, Medical School, National and Kapodistrian University of Athens, Greece
[4]e-NIOS Applications Private Company, Kallithea, Greece
[5]Department of Computer Science and Biomedical Informatics, University of Thessaly, Lamia, Greece
[6]Datamed SA, Athens, Greece
[7]Department of Digital Systems, School of Information and Communication Technologies, University of Piraeus, Piraeus

Cutaneous melanoma (CM) is the most aggressive type of skin cancer, presenting a continuous increase in its incidence rates. CM development is a complex, multi-factorial process involving the interplay of genetic and environmental risk factors. The constant progression of Next Generation Sequencing (NGS) technologies has revolutionised their applications in basic, applied, and clinical research especially in the field of oncology. Here we present part of the results from the collaborative project TRANSITION which aims at the holistic description of CM. In particular, we focus on the molecular analysis of primary melanomas from 33 patients, with detailed clinical records, including histopathologic and dermoscopic characterisation. Whole exome and RNA sequencing was performed, in order to derive their mutational landscape and expression profile. Our framework of NGS data processing integrates functional and pathway analyses, incorporating information from relevant knowledgebases, for the inference of gene signatures as candidate genes with causative role in melanoma. Specifically, we used FFPE specimens to isolate DNA and RNA from areas enriched in cancer cells and paired blood samples for germline DNA isolation, having a total of 31 paired samples for WES and 13 samples for RNA-seq (33 different patients). Our analysis was directed towards identifying melanoma susceptibility SNPs, somatic mutations and specific mutational patterns, along with melanoma-related alterations in gene expression. In this work, we also present a classification algorithm system architecture, which can integrate high-volume molecular -omic and imaging data with relevant clinical observations, in conjunction with demographic and electronic health record data, for the multi-angled description of melanoma. Ultimate aim is to develop a composite signature, which will be constantly updated through the incorporation of new cases, allowing the accurate patient classification, towards a precision medicine approach.

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P28

Novel protein-coding and non-coding transcripts of BCL2-ovarian killer (*BOK*) support its multifaceted role in human cells

Pinelopi I. Artemaki[1], Paraskevi Karousi[1], Ioannis P. Trougakos[2], Andreas Scorilas[1], Christos K. Kontos[1,*]

[1] *Department of Biochemistry and Molecular Biology, Faculty of Biology, National and Kapodistrian University of Athens, Athens, Greece*

[2] *Department of Cell Biology and Biophysics, Faculty of Biology, National and Kapodistrian University of Athens, Athens, Greece*

BCL2-ovarian killer (*BOK*), a member of the *BCL2* family of apoptosis-related genes, is a protein-coding gene with an ambiguous role. BOK is considered as a pro-apoptotic factor; however, experimental evidence suggests also an anti-apoptotic role in some instances, as well as several other functions. The splicing pattern of primary *BOK* transcripts is rather unexplored, yet its elucidation could assist in the clarification of its role in both physiological and pathological states. Therefore, we decided to study the *BOK* splice variants in a large panel of human cell lines of distinct tissue origin. For this purpose, total RNA from 39 human cancer cell lines and a non-cancerous one was isolated and reversely transcribed. Nested PCR was next conducted. After agarose gel electrophoresis, the bands of unexpected size were gel-extracted, purified, and sequenced. Finally, extensive *in silico* analysis of the novel transcripts was performed. Eleven novel *BOK* transcripts and one formerly detected in pre-eclampsia placental tissue samples were identified in the vast majority of these cell lines. Exon 2, which is considered to bear the translation start codon of the main transcript, was absent in all of these novel transcripts. In fact, 6 of them have a putative ORF, the translation of which probably leads to 2 novel BOK protein isoforms, with different structure and characteristics, compare to the already characterized isoform. The other 6 *BOK* transcripts are unlikely to encode polypeptides and may hence represent long non-coding RNAs with potential regulatory role(s). More specifically, they could antagonize with the main transcript for particular RNA-binding proteins, thus altering the *BOK* transcript ratio in cancer cells. Moreover, we discovered a novel 5'-untranslated region (5'-UTR) and 2 new 3'-UTRs; these findings suggest much more complex regulation of *BOK* transcription and translation. In summary, our study provides evidence further supporting the multifaceted role of *BOK*.

P29

The functional RNA facet of West Nile virus small RNAs

Maria Bampali [1], Adamantia Kouvela [1], Katerina Kassela [1], Nikolas Dovrolis [1], Martina Samiotaki [2], Ioannis Karakasiliotis [1]

[1]Laboratory of Biology, Department of Medicine, Democritus University of Thrace, Alexandroupolis, Greece

[2]Proteomics Facility, BSRC «Alexander Fleming», Athens, Greece

Alteration of RNA-binding Proteins (RBPs) functions have been studied during the replication of numerous viruses focusing on their role as trans-acting elements of the viral RNA(s). However, aberration from the normal function of an RBP results in the differential expression of the cellular mRNAs that it normally controls. Positive strand RNA viruses such as West Nile virus (WNV) are potent regulators of cellular post-transcriptional control as they require its effectors for an efficient RNA replication. On the other hand, viruses may generate RNAs that have functional roles. WNV accumulates a set of small RNAs called sfRNAs (subgenomic flavivirus RNAs) that are precise degradation products of the WNV genomic RNA as a result of XRN1 exonuclease activity. The exact nature and function of these sfRNAs is mostly unknown. In our study we investigated the biosynthesis of these sfRNAs and the RBPs that are regulated by WNV replication, during their recruitment on both cellular mRNAs and viral sfRNAs. Comparison of the cognate RBPs showed differences in RBP usage, possibly due to the presence of variable RNA elements on the sfRNAs that are not present in any host mRNA. Finally, as part of the recruited RBPs are proteins that induce RNA-editing on viral genome, changes in the permutational profile of host and WNV sequences may show a potential for in-host virus evolution.

P30

Evaluating the effect of JAK/STAT pathway inhibition in the functional properties of breast cancer cells with different ERα-status

Dimitris Kokoretsis[1,*], Evangelia Konstantina Maniaki[1,*], Kyriaki Tzaferi[1,*], Konstantina Kyriakopoulou[1], Christos Koutsakis[1], Zoi Piperigou[1,2], Nikos K. Karamanos[1]

[1] Biochemistry, Biochemical Analysis & Matrix Pathobiology Research Group, Laboratory of Biochemistry, Department of Chemistry, University of Patras, Patras, Greece

[2] Foundation for Research and Technology-Hellas (FORTH), Patras, Greece

[*] These authors contributed equally

Breast cancer is the most common malignancy that affects female population and is characterized by great heterogeneity. Only in 2020, 2.3 million new cases of breast cancer and 685.000 related deaths were reported globally. Estrogens and their receptors (ERs) play an important role in the development and progression of the disease. Moreover, several intracellular signaling pathways further facilitate cancer cell behavior. Of note, Janus kinases (JAK) are able to initiate a signaling cascade via signal transducer and activator of transcription (STAT), an early tumor diagnostic marker, to promote breast cancer. Recent clinical and preclinical data indicate the involvement of overexpressed and constitutively activated STAT3 in the progression, proliferation, metastasis and chemoresistance of breast cancer. In the current study, we focused on the impact of JAK/STAT signaling pathway in two breast cancer lines with different ERα status, the highly aggressive MDA-MB-231 ($ER\alpha^-$) and the non-invasive MCF7 ($ER\alpha^+$). Our results showed that inhibition of JAK2/STAT3 signaling triggers alterations in the functional properties and gene expression of critical ECM components in breast cancer cells, thus highlighting the crucial role of this pathway in the progression of breast cancer and revealing new possible markers for pharmaceutical targeting.

P31

The role of apolipoprotein E and lipid transporter ABCA7 in the pathogenesis of Alzheimer disease

Asimina Micha[1], **Christina Mountaki**[1], **Angeliki Chroni**[1]
[1]*Institute of Biosciences and Applications, National Centre for Scientific Research "Demokritos", Agia Paraskevi, Athens, GREECE*

Alzheimer's disease (AD) is a devastating neurodegenerative disease. The production and deposition of amyloid beta peptide (Aβ) is associated with AD pathogenesis. Brain cholesterol homeostasis can affect Aβ metabolism. The main lipid transport protein that maintains cholesterol homeostasis in brain is apolipoprotein E (apoE). ApoE has three common isoforms (apoE2, apoE3, apoE4), while apoE4 is the strongest genetic risk factor for AD. We showed previously that lipoprotein-associated apoE (Lp-apoE) isoforms promote the increase of Aβ production in human neuroblastoma SK-NSH cells, with an apoE4 ≥ apoE3 > apoE2 potency rank order, while carboxyl-terminal truncation to residue 166 in apoE4 prevents apoE4 from increasing Aβ levels. The cholesterol transporter ATP-binding cassette transporter A7 (ABCA7) is highly expressed in brain and ABCA7 variants have been associated with increased risk for AD. Here, we examined whether the capacity of Lp-apoE isoforms and Lp-apoE4-165 to affect differently Aβ production is associated with a different capacity to promote ABCA7-mediated cholesterol efflux. Additionally, we investigated whether the expression of ABCA7 affects Aβ production in the absence or presence of Lp-apoE4 in SK-N-SH cells. We showed that all three Lp-apoE isoforms displayed a similar capacity to promote cholesterol efflux mediated by wild-type (WT) ABCA7 in SK-N-SH cells, while Lp-apoE4-165 displayed a 50% reduction in ABCA7-dependent cholesterol efflux. The expression of WT ABCA7 in SK-N-SH cells incubated in the absence or presence of Lp-apoE4 resulted in the reduction of extracellular Aβ production. Overall, our data indicate that WT ABCA7 can have a direct effect on Aβ production, independently of its capacity to interact with Lp-apoE and promote cholesterol efflux in neuronal cells. Further studies are underway on the elucidation of the mechanism of Aβ levels reduction by WT ABCA7, the effect of pathogenic ABCA7 variants on AD-related processes and the interaction of apoE forms with pathogenic ABCA7 variants.

P32

Expression and function of CYLD in kidney tumor cells

Dimitris Papanikolaou[1], Dimitris Koutsoubaris[2], Elena Constantinou[2], Dimitris Karatzas[3], Eudoxia G. Hatzivassiliou[2*]

[1] Second Department of Urology, School of Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece

[2]School of Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, University campus, 54124 Thessaloniki, Greece

[3] Department of Genetics, Developmental and Molecular Biology, School of Biology, Aristotle University of Thessaloniki, University campus, 54124 Thessaloniki, Greece

**Corresponding author,Eudoxia G. Hatzivassiliou, School of Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, University campus, 54124 Thessaloniki, Tel: +302310999105,e-mail: eudoxiah@auth.gr*

Introduction The tumor suppressor gene Cyld has been of particular interest recently, due to its involvement in many forms of neoplasia including head and neck squamous cell carcinoma multiple myeloma, melanoma, colon adenocarcinoma, hepatocellular carcinoma, carcinomas of breast, pancreas and lung. The protein CYLD is a cytoplasmic enzyme with deubiquitinase properties. CYLD selectively hydrolyzes polyubiquitin chains, which are not involved in the degradation of proteins through the proteasome, but act as scaffolds to form signalling regulatory complexes. The protein CYLD is involved in the regulation of signalling pathways associated with cell division, cell survival and the human immune system. Studies on the molecular function of CYLD have shown that it interferes with NF-kappaB, JNK, p38 and Wnt signaling.

Objectives The present study aims to investigate the hypothesis that downregulation of the tumor suppressor protein CYLD is implicated in oncogenesis of clear cells, in renal clear cell carcinoma (ccRCC).

Materials and Methods Towards this goal, immunohistochemistry and Real Time PCR experiments from formalin fixed paraffin embedded (FFPE) tissue blocks from ccRCC patients were performed, in order to analyze CYLD expression in clear cell renal cell carcinoma and matched normal tissue specimens. In addition, a clonogenic assay was performed in order to analyze the effect of CYLD wt and a catalytically inactive mutant CYLD on the growth of human embryonic kidney cells.

Results In the present study the results show that CYLD is downregulated at protein and mRNA level in patients with ccRCC. This is further corroborated by the results of a clonogenic assay, which showed a deubiquitinating activity-dependent growth inhibitory role of CYLD in human embryonic kidney cells.

Conclusion Our results support the notion that CYLD can have a tumor suppressing role at least in a subset of clear cell renal cell carcinoma, suggesting that it can be incorporated in the future in the development of targeted therapeutic approaches.

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P33

Analysis of dsRNA production during baculovirus infection

Luc Swevers[1], Anna Kolliopoulou[1,2], Dimitrios Kontogiannatos[1], Aleksander Józef Mazurek[1,2], Izabela Prifti[1,2], Vasiliki-Maria Christopoulou[1] and Vasiliki Labropoulou[1]

[1] *Insect Molecular Genetics and Biotechnology, Institute of Biosciences & Applications, National Centre for Scientific Research "Demokritos", Aghia Paraskevi, 15341 Athens, Greece*

[2] *Department of Biomedical Sciences, University of West Attica, 12243 Athens, Greece*

The baculovirus expression vector system (BEVS) is a useful platform for the expression of recombinant proteins and the production of large protein complexes such as viral-like particles (VLPs), which can be used for the targeted delivery of drugs or toxins. However, methods need to be developed to obtain efficient loading with the intended cargo. Our research intends to employ the BEVS for the production of VLPs to deliver insecticidal dsRNAs to targeted insect pests. A convenient strategy would be the co-expression of long dsRNAs with viral capsid proteins concomitantly with their encapsulation during VLP assembly, but the capacity of the BEVS for the production of long dsRNA has not been assessed so far. Here, the efficiency of production of long RNA hairpins targeting the *luciferase* gene by the *polyhedrin* promoter during baculovirus infection was evaluated. However, RNAi reporter assays could not detect significant amounts of dsLuc in Hi5 cells infected with recombinant baculovirus, even in the presence of co-expressed dsRNA-binding protein B2-GFP or the employment of the MS2-MCP system. Nevertheless, dot blot analyses using anti-dsRNA antibody revealed that baculoviral expression of B2-GFP significantly increased the dsRNA levels in infected cells that may correspond to hybridized complementary viral transcripts. Using B2-GFP as a genetically encoded sensor, dsRNA foci were detected in the nuclei that partially co-localized with DAPI staining, consistent with their localization at the virogenic stroma. Co-localization experiments with specific baculoviral proteins indicated limited overlap between B2-GFP and the ring zone compartment, where assembly of nucleocapsids and virions occurs. Exogenous dsRNA proved to be strongly resistant to degradation in stability experiments where extracts of non-infected and infected Hi5 cells were used, and it is proposed that strong unwinding activity in the infected nuclei may neutralize the annealing of complementary RNA strands and block the production of long dsRNAs.

P34

The role of sulfated hyaluronan on the functional properties and matrix effectors expression of breast cancer cells with different ER status

Christos Koutsakis[1], Anastasia-Gerasimoula Tavianatou[1], Dimitris Kokoretsis[1], George Baroutas[1], Nikos K. Karamanos[1,2]

[1]Biochemistry, Biochemical Analysis & Matrix Pathobiology Research Group, Laboratory of Biochemistry, Department of Chemistry, University of Patras, Greece

[2] Foundation for Research and Technology-Hellas (FORTH) / Institute of Chemical Engineering Sciences (ICE-HT), Patras, Greece

Breast cancer constitutes one of the most common malignancies, with the expression patterns of estrogen receptors (ERs) being crucial during disease development. Depending on the ER status, breast cancer can be categorized into different types. The ERα(+) types are characterized as less invasive and are correlated with better prognosis in comparison to the ERα(-) types, which are more aggressive and metastatic. Hyaluronan (HA) is an extracellular matrix glycosaminoglycan (GAG) that plays a pivotal role in a variety of biological processes. Its elevated levels have been correlated with breast cancer aggressiveness and poor prognosis. While HA is the only GAG not normally substituted with sulfate groups, sulfated hyaluronan (S-HA) has previously been used in scientific studies with promising results. These studies demonstrate that S-HA fragments can induce apoptosis on prostate and bladder cancer cells and inhibit their motility and invasiveness. The aim of the present study was to evaluate the effects S-HA fragments have on breast cancer cells with different ER status. To this end, the ERα(+) MCF-7 cells and the ERα(-)/ERβ(+) MDA-MB-231 cells were used. The cells were treated with both nonS-HA fragments and S-HA fragments of 50 kDa. Proliferation, wound healing, adhesion and invasion assays were performed in order to determine the effects HA fragments have on the cells' functional properties. The expression of matrix effectors was analysed at a gene level using real-time PCR, while the cell morphology and expression of EMT markers was observed with phase-contrast microscopy and immunofluorescence respectively. According to the results, S-HA attenuates breast cancer cell proliferation, migration and invasion, while it increases their adhesion on collagen type I. Notably, the S-HA fragments seem to exhibit a stronger effect on these properties compared to the one mediated by the nonS-HA 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 levels for matrix effectors and EMT markers, such as MMP2, MMP9, MT1-MMP, HAS2/3 and E-Cadherin, SNAI2/Slug. Consequently, a deeper understanding of the mechanism by which the S-HA fragments orchestrate these processes could contribute to the development of therapeutic strategies.

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P35

Comorbidities Risk Assessment: a predictive approach

Angeliki Balasopoulou[1#], Jose Carlos Gomez Tamayo[2#], Theodora Katsila[1*]
[1]*Institute of Chemical Biology, National Hellenic Research Foundation, Athens, Greece*
[2]*Research Programme on Biomedical Informatics (GRIB), Hospital del Mar Medical Research Institute (IMIM), Department of Experimental and Health Sciences, Universitat Pompeu Fabra, Barcelona, Spain,*
[#]*These authors contributed equally to this work*
**email: thkatsila@eie.gr*

Complex disease traits and clinical phenotypes stem from genetic and environmental influences (e.g. diet, viral biomolecules/stimuli). Problematic self-regulation of the inflammasome may serve as a paradigm. Inflammatory responses shift a defense mechanism into a perpetuating inflammatory response leading to disease comorbidities. Herein, we developed a predictive approach for comorbidities risk assessment that highlight molecular signatures of disease severity.
An in-depth *in silico* analysis of disease traits and clinical phenotypes as well as tissue-specific datasets were integrated with functional data from publicly available data repositories and databases. R and Python programming languages were employed. Two strategies were implemented: agnostic analysis and targeted analysis, followed by extensive data and text mining for extra filtering of outcomes. Protein networks revealed missense variants of interest. Data and text mining were performed to account for biases.
Our approach detected patterns of comorbidities as the net result of genetic and environmental influences (e.g. diet, viral biomolecules/stimuli). *In silico* analyses resulted in 1,156 missense variants of key interest either at the orthosteric or allosteric site of key proteins.
A predictive evaluation of comorbidity development risk may identify those patients at high risk and thus, empower optimum patient stratification

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P36

Targeted long-read sequencing reveals a wide variety of circular *BCL2L12* transcripts and novel aspects of circular RNA (circRNA) biology

Paraskevi Karousi, Diamantis C. Sideris, Andreas Scorilas, Christos K. Kontos*
Department of Biochemistry and Molecular Biology, Faculty of Biology, National and Kapodistrian University of Athens, Athens, Greece

Circular RNAs (circRNAs) represent transcripts resulting form back-splicing. These transcripts were initially considered splicing by-products until advances in high-throughput sequencing uncovered their widespread expression. However, knowledge regarding circRNAs deriving from apoptosis-related genes is still limited. *BCL2L12* is a member of the BCL2 family, widely expressed in colon cancer. Although BCL2L12 protein does not bear a typical anti-apoptotic structure, it has been reported to exert anti-apoptotic function. In this study, we aimed at identifying novel *BCL2L12* circRNAs, using targeted long-read sequencing. Total RNA extracts from Caco-2, COLO 205, DLD-1, RKO, HT-29, HCT 116, and SW 620 colon cancer cell lines were reversely transcribed, using random hexamers. First-round PCR was conducted using divergent primers for each *BCL2L12* exon, followed by nested PCR. Libraries for long-read sequencing were then prepared, and nanopore sequencing was performed using the MinION Mk1C platform and the Flongle adapter. Long-read sequencing data analysis was performed, using publicly available programs (Minimap2, TranscriptClean, and Samtools) and our own Perl-based scripts. This experimental approach revealed that a wide variety of circRNAs can be transcribed by a single gene, as 62 novel *BCL2L12* circRNAs were identified. Novel *BCL2L12* exons were also detected, as well as several extensions of the currently annotated exons. Most part of the *BCL2L12* gene is present in distinct circRNA sequences. Additionally, we observed that back-splicing may occur between cryptic exons and micro-exons that share short sequence similarity, supporting the notion of a novel back-splicing mechanism. Surprisingly, poly(A) stretches were found in *BCL2L12* circRNAs, although circRNAs have been defined so far as transcripts lacking poly(A) tails. This study not only revealed the existence of a wide variety of *BCL2L12* circRNAs, but also highlighted novel aspects of circRNA biology, providing evidence about unknown mechanism(s) of circRNA biogenesis. Moreover, the regulatory and protein-coding potential of these novel circRNAs merits further investigation.

P37

Proteomic analysis of mouse kidney tissue associates peroxisomal dysfunction with early diabetic kidney disease.

Aggeliki Tserga[1], Despoina Pouloudi[2], Jean Sébastien Saulnier-Blache[3], Rafael Stroggilos[1], Irene Theochari[2], Harikleia Gakiopoulou[2], Harald Mischak[4], Jerome Zoidakis[1], Joost Peter Schanstra[3], Antonia Vlahou[1], Manousos Makridakis[1*]

[1.] *Biomedical Research Foundation, Academy of Athens, Department of Biotechnology, 11527 Athens, Greece*

[2.] *1st Department of Pathology, School of Medicine, National and Kapodistrian University of Athens, Greece*

[3.] *Institut National de la Santé et de la Recherche Médicale (INSERM), U1048, Institut of Cardiovascular and Metabolic Disease, Toulouse, France, Université Toulouse III Paul-Sabatier, Toulouse, France*

[4.] *Mosaiques Diagnostics GmbH, Hannover, Germany*

*Corresponding author: Manousos Makridakis, PhD

Email: mmakrid@bioacademy.gr

Background: The absence of efficient inhibitors for DKD progression reflects the gaps in our understanding of DKD molecular pathogenesis. A comprehensive proteomic analysis was performed on glomeruli and kidney cortex of diabetic mice with subsequent validation of findings in human biopsies and -omics datasets aiming to better understand the underlying molecular biology of early DKD development and progression.

Methods: LC–MS/MS was employed to analyze the kidney proteome of DKD mouse models: Glomeruli of Ins2Akita mice 2 month and 4 month old, and cortex of db/db mice 6 month old. Following label-free quantification, the abundance of detected proteins were correlated with existing kidney datasets and functionally annotated. Tissue sections from 16 DKD patients were analyzed by IHC.

Results: Pathway analysis of differentially expressed proteins in the early and late DKD versus controls predicted dysregulation in DKD hallmarks (such as peroxisomal lipid metabolism, β-oxidation and TCA cycle) supporting the functional relevance of the findings. Comparing the observed protein changes in early and late DKD, consistent upregulation of 21 and downregulation of 18 proteins was detected. Among these were downregulated peroxisomal proteins such as NUDT19, ACOX1, and AMACR and upregulated mitochondrial proteins related to aminoacid metabolism including GLS, GLDC, and GCAT. Several of these changes were also observed in the kidney cortex proteome of db/db mice. IHC of human kidney further confirmed the differential expression of NUDT19, AGPS, AMACR and CAT proteins in DKD.

Conclusions: Our study shows an extensive differential expression of peroxisomal proteins in the early stages of DKD that persists regardless of the disease severity. These proteins therefore represent potential markers of early DKD pathogenesis. Collectively, essential pathways associated with peroxisomes such as lipid β-oxidation, plasmalogen synthesis, aminoacid metabolism and response to oxidative stress are downregulated in early DKD, providing new perspectives and potential markers of diabetic kidney dysfunction.

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P38

Biochemical characterization of novel Autotaxin inhibitors identified via a newly established drug discovery pipeline

Elli-Anna Stylianaki[1], Christiana Magkrioti[1], Ladopoulou Eleni[2], Georgia Melagraki[3], Antreas Afantitis[3], Alexios Matralis[1], Vassilis Aidinis[1].
[1]Institute of Bioinnovation, Biomedical Sciences Research Centre "Alexander Fleming", Vari, Greece
[2]Biology Department, National and Kapodistrian University, Athens, Greece
[3]Novamechanics Ltd, Nicosia, Cyprus

Autotaxin (ATX) is an enzyme, omnipresent in biofluids, that converts Lysophosphatidylcholine (LPC) to Lysophosphatidic acid (LPA). Its broad effect is mediated by the binding of LPA to its respective G-protein coupled receptors (LPAR1-6), which are expressed in the vast majority of cell types. Pathological increase of ATX levels has been implicated in the progression of various inflammatory and fibroproliferative diseases, such as Idiopathic Pulmonary Fibrosis and Non-alcoholic Steatohepatitis, while genetic and pharmacological attenuation of its activity was shown to abrogate these diseases' manifestation. This suggested ATX as a valuable drug target, a fact appreciated from both the academic sector and the pharmaceutical industry, although this effort has not yet led to the discovery of a safe and potent inhibitor. In our attempt to develop novel ATX inhibitors, we selected the crystal structure of ATX in complex with a nanomolar-range efficient antagonist as a docking reference so as to *in silico* screen the publically available HitFinder small molecule database. This yielded a list of 30 initial candidates which were further structurally optimized with chemoinformatic tools. Eventually, the most promising molecules were *in vitro* screened by employing a well-established enzymatic activity assay. This screening identified SC-49 as the most prominent inhibitor with the best drug-like and synthetic properties. Therefore, we performed a Structure-Activity Relationship study for this molecule, leading to the identification of two compounds that exhibit an IC50 at the lowest micromolar range. Both their aqueous solubility and cytotoxicity were studied as well as their mode of inhibition. In addition to this, their molecular docking and molecular dynamics profile was explored, allowing a deeper understanding of their binding mechanism to ATX. Conclusively, our endeavor resulted to the development of these two novel lead compounds via a newly established drug discovery pipeline assisted by chemoinformatic tools and *in vitro* assays.

P39

NMR interaction studies of Arkadia's RING domain with two independent E2 enzymes

Tamara Toro[1], Maria Birkou[1], Georgia N. Delegkou[1], Konstantinos D. Marousis[1], Vasso Episkopou[2], Georgios A. Spyroulias[1*]
[1]*University of Patras, Greece*
[2]*Imperial College, London, United Kingdom*

Ubiquitination is an essential mechanism for the regulation of cellular physiology. Ubiquitin molecules can be covalently attached to a target protein on single site (monoubiquitinating), multiple sites (multi-monoubiquitinating) or on poly-ubiquitin chains. The linkage of lysine residues determines the final structure and signaling of the ubiquitin chains. The ubiquitination cascade involves three enzymes: 1) an activating enzyme (E1), a conjugating enzyme (E2) and an ubiquitin ligase (E3), which act sequentially[1].
Arkadia/RNF111 is an E3 ligase, which consists of 994 amino acids in humans. Arkadia functions as an E3 ubiquitin ligase through its C-terminus RING-H2 domain (residues 942-983). The RING-H2 domain is a key player for the specificity and the recruitment of E2 enzymes. Arkadia positively regulates the Transforming Growth Factor-β (TGF-β) signaling pathway by promoting ubiquitin-dependent degradation of the molecules Smad2/3, Smad7, SnoN and Ski. The enzymatic activity of Arkadia on TGF-β mainly depends on its interaction with the E2 enzyme UbcH5b[2]. Additionally, Arkadia is implicated in the DNA damage response by acting as SUMO-targeted ubiquitin ligase (STUbL). More specifically, Arkadia interacts with the heterodimer complex of UbcH13-MMS2 and promotes K63 polyubiquitination of SUMOylated proteins as XPC (Xeroderma Pigmentosum C). The presence of MMS2 is essential for K63-linked ubiquitin chains synthesis[3].
The aim of this study is to identify the Arkadia amino acid segments which are crucial determinants of its E2 selectivity and effective interaction in ubiquitination process. Specifically, what is presented herein is the interaction of the RING domain of Arkadia with the E2 enzyme UbcH13, monitored by NMR and the NMR mapping of the interaction interface of Arkadia-UbcH13 and Arkadia-UbcH5b complexes.

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STUDY ON PHOTOSYNTHESIS OF RECOMBINANT CYANOBACTERIA LACKING PHYCOCYANIN

Aikaterini Yiakoumidaki-Voyiatzi[1*], Elisavet Amanatidou[2], Anastasios Melis[3], Kostas Stamatakis[1]
[1]Biophysics and Biotechnology of Membranes Laboratory, Institute of Biosciences and Applications, N.C.S.R. "Demokritos", Patr. Grigoriou E & Neapoleos 27, 153 41, Ag. Paraskevi, Athens, Greece
[2]Department of Chemical Engineering, University of Western Macedonia, 501 00, Koila, Kozani, Greece
[3]Department of Plant & Microbial Biology, University of California, Berkeley, CA 94720-3102, USA
**katerina.giakoumidaki24@gmail.com*

The main light-harvesting antenna of cyanobacteria is the phycobilisome(PBS) and it is responsible for the optimal function of the photosynthetic apparatus, by redistributing the excitation energy between Photosystem(PS)II and PSI. In Synechocystis sp. PCC 6803(S6803) cells, the PBS consists of the phycobiliproteins phycocyanin(PC), peripherally, and allophycocyanin, centrally. Heterologous production of isoprene hydrocarbons in cyanobacteria, emanating from their photosynthesis, is attracting increasing attention. The approach entails the heterologous expression of isoprene synthase(IspS) from terrestrial plant Pueraria montana, in Synechocystis sp. PCC 6803 cells, thus providing the last step for isoprene biosynthesis in the MEP pathway. The IspS gene is fused with the cpcB gene, which codes for the highly-expressed β-subunit of PC. The following strains were constructed: cpcB*L7*IspS[1], cpcB*L7*IspS+Fni[2](absence of PC, able to produce isoprene). The recombinant strains lack PC, resulting in a truncated structure of the PBS. A way to assess the regulation of the excitation energy transfer of the recombinant strains is by low-temperature (77K) fluorescence emission spectra. In addition, the following are being used as control strains: S6803(wt) and Δcpc[1](deletion of cpc operon). First results indicated that Chls a of the recombinant strains are capable of transferring energy between the PSs. To test this observation, new measurements were conducted in the presence of the DBMIB inhibitor, which prevents re-oxidation of the PQ-pool. The results were positive of our assumption that Chls a are indeed capable of transferring energy between the PSs, without the interference of the truncated PBS.

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P41

Autotaxin (ATX) is present on immune cell subpopulations of BALF during bleomycin-induced pulmonary fibrosis

Konstantinos Ntatsoulis[1], Apostolos Galaris[1], Petros Prapas [1] and Vassilis Aidinis[1]
[1] Institute of Bio-Innovation, Biomedical Sciences Research Center Alexander Fleming, 16672 Vari, Attica, Greece

Idiopathic Pulmonary Fibrosis (IPF) is a chronic, fatal interstitial pneumonia characterized by excessive extracellular matrix (ECM) deposition and disruption of pulmonary architecture. The etiology of IPF is largely unknown; yet the immune system appears to be involved in the pathophysiology of the disease and especially macrophages (MΦs). Alveolar MΦs, known for their homeostatic role in the lung, as well as the monocyte-derived MΦs have been shown to regulate fibrotic responses in the lung via the production of various factors and chemokines. Previous studies of our lab have shown that Autotaxin (ATX), a secreted enzyme important for generating LPA, has a role in pulmonary fibrosis pathophysiology and molecules targeting ATX are already in clinical trials against IPF.
In this study we established a FACS gating strategy that can accurately discriminate and monitor the kinetics of the major immune cell populations in bronchoalveolar lavage fluid (BALF) during the disease progression of the bleomycin-induced mouse model of pulmonary fibrosis. Also, it allows the accurate identification of ATX in the surface of these cells. We observed major differences in the distribution of the immune cells during the disease progression. Finally, ATX appears to be present on the surface of all MΦs subpopulations and a small portion of T cells, in the fibrotic stage of the disease. Our work could contribute to the understanding of the role of both the immune cells and ATX in the pathophysiology of pulmonary fibrosis.

P42

Tau protein as a regulator of neurogenic brain plasticity under chronic stress

Chrysoula Dioli[1,2], Patricia Patricio[2], Nuno Sousa[1,2], Luisa Pinto[2], Ioannis Sotiropoulos[1,2]
[1] *Institute of Biosciences and Applications, NCSR "Demokritos", Athens, Greece*
[2] *Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal*

Tau is a cytoskeletal protein involved in many cellular processes such as neuronal differentiation, axonal growth and synaptic plasticity while Tau hyperphosphorylation has been causally related to neuronal atrophy/dysfunction, diminished neurogenesis and memory loss that characterize Alzheimer's disease (AD). Chronic stress, a well-known "sculptor" of adult brain plasticity, reduces hippocampal neurogenesis but the exact underlying cellular cascades are poorly investigated. Despite the well-known role of Tau in regulating cytoskeletal dynamics and the suggested relationship of hyperphosphorylated Tau with cytoskeletal damage, the involvement of Tau in damage of neurogenic brain plasticity caused by chronic stress remains poorly explored. Herein, we demonstrate that chronic stress triggers Tau hyperphosphorylation and 4R-Tau/3R-Tau imbalance in newborn cells and immature neurons of the adult brain via the PI3K/mTOR/GSK3β/β-catenin signaling, known to regulate cell survival and proliferation. Moreover, deletion of Tau attenuated the stress-driven neurogenic, but not astrogliogenic or oligodendrogenic, damage in the cytogenic niches of the adult brain (hippocampus and subventricular zone-olfactory bulb system) indicating the neuronal-specific involvement of Tau in the stress-driven cytogenic damage of the adult brain. We also monitor the impact of stress on dendritic maturation of immature neurons demonstrating for the first time that chronic stress triggers opposite neuroplastic effect on different dendritic compartments of the same immature neuron. In summary, the above studies suggest one cell-autonomous and one non-cell autonomous mechanism through which chronic stress damages neurogenic plasticity in different areas of the adult brain, adding to our limited knowledge of how stressful conditions precipitate brain pathology.

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P43

Interaction between the Circadian Clock and Pathogen-Triggered Immunity in *Phaseolus vulgaris*

Angeliki Galeou, Chrysanthi Stefanatou and Anastasia Prombona*
Institute of Biosciences and Applications, NCSR 'Demokritos', 15341 Agia Paraskevi, Greece
**e-mail: prombona@bio.demokritos.gr*

The circadian clock is the endogenous timekeeping mechanism of all living organisms that synchronizes physiology and metabolism to the 24-hour cycles of day/night changes. Additionally, in plants, the clock has been shown to play a major role in controlling the defence mechanisms during microbial attack but also to receive significant feedback from the infection process. The present work is the first study that investigates the crosstalk of the circadian clock and the pathogen-triggered immunity (PTI) in the common bean caused by one of the plant's major pathogens, *Pseudomonas syringae pv. phaseolicola* (Psp). Our analysis during the initial stages of infection of Red kidney bean leaves with a virulent Psp strain included assays of *in planta* bacterial growth, of the oxidative burst induced by the bacterial elicitor peptide flagellin 22 and the expression of genes involved in the local defence response. Our experiments showed a temporal variation of the defence responses with circadian characteristics. Additional experiments aim to uncover the regulatory role of the circadian clock in the PTI-relevant defence mechanisms.

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P44

Contribution of MAPK pathways in matrix metalloproteinase induction during *in vitro Helicobacter pylori* infection.

Yiannis Karayiannis[1,2], Eleftherios Kontizas[1,2], Beatriz Martinez-Gonzalez[1], Panagoula Kollia[2], Andreas Mentis[1], Dionyssios Sgouras[1]*

[1]*Laboratory of Medical Microbiology, Hellenic Pasteur Institute, Athens, Greece*

[2]*Department of Genetics and Biotechnology, Faculty of Biology, School of Physical Sciences, University of Athens, Athens, Greece*

**email: sgouras@pasteur.gr*

Chronic inflammatory response to persistent *Helicobacter pylori* (*Hp*) infection remains the primary risk factor for gastric neoplasia. Remodeling of extracellular matrix in gastric adenocarcinoma development is mediated by proteolytic activity of Matrix Metalloproteinases (MMPs) MMP-3, MMP-7, MMP-9 and MMP-10, among others. Aberrant expression and involvement of these MMPs in Epithelial-to-Mesenchymal transition (EMT) has been reported in both *in vitro* and *in vivo* models of *Hp* infection. Furthermore, upregulation of stromelysins MMP-3 and MMP-10, matrilysin MMP-7 and gelatinase MMP-9 have been associated with the expression, endocytic translocation and tyrosine phosphorylation state of the *Hp* CagA oncoprotein in gastric epithelial cells.

In the present study we aim to assess the contribution of MAPK signaling in the CagA-dependent activation of MMP-3 and MMP-9. To this end, human gastric epithelial cell lines (AGS & GES-1) were co-cultured with isogenic CagA-positive *Hp* strains, in the presence of inhibitors of JNK, ERK1/2 and p38 MAPK pathways. Transcriptional and protein expression levels of MMP-3 and -9, EMT factors ZEB-1, and Snail and stemness factor CD44, were assessed by qPCR and Western blot.

Hp infection alone resulted in elevated levels of MMP-3 and MMP-9 in both cell lines. In AGS cells, inhibition of ERK1/2 decreased transcription levels of both MMPs, to levels comparable to the uninfected control and JNK and p38 inhibitors resulted in upregulated mRNA levels of both MMP genes. Moreover, infection in the presence of each inhibitor decreased MMP-3 protein expression to constitutive levels, however MMP-9 protein expression was found downregulated only in the presence of JNK or ERK1/2 inhibitors. ZEB-1, Snail and CD44 were upregulated in a CagA-dependent manner and inhibition of p38 pathway resulted in their upregulated gene expression. In GES-1 cells, inhibition of JNK or ERK1/2 pathway induced downregulation of both MMPs expression, while blocking of p38 cascade did not. Further analysis relating to the induction of EMT is under way.

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P45

^{1}H-NMR based metabolomic analysis of preterm infants with necrotizing enterocolitis (NEC)

Panagiota D. Georgiopoulou[1], Styliani A. Chasapi[1], Irini Christopouloou[2], Anastasia Vargarigou[2], Georgios A. Spyroulias[1]

[1]*Department of Pharmacy, University of Patras, 26504 Patras, Greece*

[2] *Department of Pediatrics, University of Patras Medical School, General University Hospital, Patras, Greece*

Preterm birth is considered as a worldwide problem in neonatology, remaining nowadays a major challenge for physicians and other healthcare providers. Every delivery that occurs before 37 weeks of pregnancy, is a preterm birth, ranging from extremely (<28 weeks) to late (32-37 weeks) preterm, according to Worldwide Health Organization (WHO). Despite the reduction of the mortality rate, prematurity has a direct impact on the newborn's health immediately after birth but also on the later life. Necrotizing enterocolitis (NEC) is a disease that causes intestine's wall inflammation leading to its destruction that mainly affects premature infants [1]. The aitiology of NEC remains poorly understood and the early diagnosis or prognosis have obstacles to overcome, since there are not specific clinical signs. Herein, there is a need for biomarkers that could shed light, leading to accurate medical treatment, reducing the mortality rate and the hospitalization time. The ever-growing potential and clinical applications of metabolomic analysis appears as a promising approach [2]. Through NMR based metabolomics, we aimed to investigate the urine metabolomic profile and reveal those metabolites which could be indicative for an initial discrimination between newborns diagnosed with NEC and the healthy ones. The acquired NMR data of six newborns, clinically diagnosed with NEC and hospitalized in Neonatal Intensive Care Unit (NICU) were compared to a group consisted of six preterms hospitalized in NICU without NEC and six healthy, preterm, newborns. Multivariate and univariate statistical analysis were implemented to NMR numerical data and revealed differences in the metabolic profiles. The outcome of the analysis presented, herein, indicates differentiation in metabolic processes and confirms the use of urine metabolomics, a non-invasive approach, to determine the health status of an individual.

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P46

Synthesis, photophysical properties and *in vitro* evaluation of novel BODIPY fluorophores

Olga Kirkilessi,[1] Christina Arapatzi,[2] Vasiliki Kostourou,[2] Kyriakos C. Prousis,[1] Theodora Calogeropoulou[1]
[1]*National Hellenic Research Foundation, Institute of Chemical Biology, Athens, Greece*
[2]*"Alexander Fleming" Biomedical Sciences Research Center, Institute for Bioinnovation, Athens, Greece*

Fluorescent dyes have emerged as a powerful tool for visualizing and labelling biomolecules *in vitro* and *in vivo*. Boron-dipyrromethenes (**BODIPYs**) consist a class of fluorophores with wide applicability, as their structural versatility makes possible to fine-tune their spectroscopic properties.[1]
Prompted by our previous work,[2] series of new mono-, di- and tri-substituted 8-phenyl BODIPY derivatives were synthesized. Our aim was to extend the π-conjugation system and to study the effect of different aromatic and heteroaromatic substituents at positions 2,3,5 and 6 on the electronic properties of the parent compound. Fluorescence spectroscopy was employed to evaluate the influence of the substituents installed on the photochemical properties of the resulting dyes in various solvents and pH values. The cellular localization and toxicity of the best candidates were assessed in non-endothelial cells at three concentrations (0.1mM, 1mM and 10mM) using confocal fluorescence microscopy.
The majority of the new dyes exhibited attractive spectroscopic properties with significant red-shifted emission (510-680 nm), suitable Stokes shifts (9-50 nm) and quantum yields (φ) (0.10-0.73) and high brightness. Furthermore, all the dyes tested were non-toxic and photostable *in vitro*. Finally, the most promising dyes were **TC498** (λexc:568 nm, λemi:590 nm) and **TC514** (λexc:590 nm, λemi:640 nm). **TC498** was localized in cell membrane structures, while **TC514** was localized at cell membrane and within minutes in the endocytic vesicles.

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P47

Dynamic re-distribution of the macroH2A chromatin landscape during cellular reprogramming rewires gene expression programs required for pluripotency

Dimitrios Valakos[1], Antonis Kokkalis[1], Alexandros Polyzos, Maria Pliatska, Fotis Kirilis, Aggelos Banos, Giannis Vatsellas, and Dimitris Thanos*
Biomedical Research Foundation Academy of Athens, 4 Soranou Efesiou Street, Athens 11527 Greece
[1] The authors contributed equally.
**Corresponding author Tel: +30 2106597244; E-mail: thanos@bioacademy.gr*

The histone variants macroH2A1 and macroH2A2 inhibit the cellular reprogramming of fibroblasts to induced pluripotency by blocking the transition from the mesenchymal to the epithelial state (MET), yet the molecular mechanisms still remain elusive. Herein, we investigated these mechanisms by performing detailed transcriptome analysis of Mouse Embryonic Fibroblasts (MEFs) undergoing reprogramming in which macroH2A1 and macroH2A2 were knocked down. These data were integrated with results from parallel ChIP-seq experiments aiming to localize the position of macroH2A-containing nucleosomes in genes whose expression is altered in the knocked-down cells. Our experiments revealed an unprecedented, massive and dynamic re-distribution of macroH2A variants throughout the genome, thus underscoring the role of macroH2A nucleosomes in the formation of the epigenetic landscape. Specifically, we found that macroH2A1-, but not macroH2A2-containing nucleosomes are enriched at promoters of expressed and non-expressed genes. We discovered that immediately upon the start of the reprogramming process, macroH2A1-containing nucleosomes depart from their target promoters and reappear on the same promoters but in a new position located 50bp upstream or downstream from the original site a few days later. We demonstrated that these nucleosome repositioning events regulate the accessibility of the transcription E2F4, a negative regulator of cell cycle progression, to its binding sites at target genes. Thus, in MEFs, E2F4 binds to its target genes and inhibits cell cycle progression. However, when the reprogramming process begins, the nearby located macroH2A1-containing nucleosomes are mobilized and are repositioned to new sites masking these E2F4 binding sites, thus preventing E2F4 from binding. Inhibition of E2F4 binding leads to a relief of cell cycle arrest, a requirement for cellular reprogramming. In summary, our studies revealed a novel mechanism for the dynamic role of specialized chromatin landscapes in the establishment and robustness of gene expression programs determining cell identity and plasticity, with implications in tumorigenesis and metastasis.

P48

RASSF1A facilitates 53BP1 in nucleolar ATM signal amplification during ribosomal DNA break repair

Stavroula Tsaridou[1], Eric O'Neill[2] and Dafni Eleftheria Pefani[1]
[1] Department of Biology, School of Medicine, University of Patras, Patras 26504, Greece.
[2] Department of Oncology, University of Oxford, Old Road Campus Research Building, OX3 7DQ, Oxford, UK.

Genome integrity and cell survival are constantly threatened by various DNA lesions across the genome. Emerging evidence support that not all genomic areas are equally susceptible to DNA damage and repair in these loci can be more challenging acting as hotspots of genomic instability. The ribosomal RNA gene repeats (rDNA) that transcribe for the ribosomal RNA are located in the five acrocentric chromosomes and are organized in the most prominent nuclear structure, the nucleolus. The clustering of the repeats, the repetitive nature, the high transcriptional activity and the formation of secondary DNA structures constitute the rDNA as a potential fragile site. Notably, recent progress in understanding how the rDNA damage response is organized has highlighted the key role of adaptor proteins in the response.
In this study we identify the tumor suppressor protein RASSF1A as a bona fide DNA repair factor of the nucleolar DNA damage response. RASSF1A is an adaptor protein that undergoes frequent promoter methylation in several tumor types including lung cancer. Employing targeted rDNA damage, we find robust recruitment of the scaffold at rDNA breaks. RASSF1A recruitment depends on ATM phosphorylation and interaction with the 53BP1 protein. RASSF1A at the sites of rDNA breaks facilitates ATM local signal amplification and promotes homology mediated repair. Downregulation of RASSF1A, an early event that takes place during malignant transformation, results in persistent breaks, rDNA copy number alterations and reduced cell survival in response to genotoxic stress. Our study offers better mechanistic insights on how the DNA damage response is organized at the nucleolus, a hub of genomic instability and further highlights the use RASSF1A as an emerging biomarker for genotoxic cancer treatments.

P49

Repression of the mevalonate pathway by statins induces the UPR^{mt} program through ISR but does not affect the protein synthesis rate

Fotini Filippopoulou, Panagiota Batzali, Dionysios Chartoumpekis and Ioannis Habeos
Department of Internal Medicine, Division of Endocrinology, School of Medicine, University of Patras, 26 504 Patras, Greece

UPR^{mt} is a transcriptional response activated by multiple forms of mitochondrial dysfunction and regulated by the mitochondrial-to-nuclear communication, inducing the expression of nuclear genes, promoting the repair and recovery of the cellular function. The main transcription factor that activates the UPR^{mt} genes is ATF4. The levels of ATF4 protein rise during the ISR through phosphorylation of eIF2α, while the total protein synthesis is inhibited. Studies on *C. elegans* have shown that UPR^{mt} depends on the mevalonate pathway, the metabolic pathway of cholesterol, isoprenoids and CoQ biosynthesis. Statins repress the mevalonate pathway through inhibition of HMG- CoA reductase enzyme. The aim of this research is to study the effects of statins on the ISR and the UPR^{mt} in mammalian cells and mouse models.

Materials and Methods: L6 and HEK293T cell lines were treated with 2.5μM Simvastatin and FCCP (10μM), an inducer of mitochondrial stress, with or without simvastatin pre- and co-treatment. 3-month-male mice were treated with Simvastatin 0.1% through food for 14 days and a part of the liver and the muscle were isolated. The relative levels of UPR^{mt} genes were measured by RT-qPCR and the levels of ATF4 by Western blotting, at both the cell lines and the tissues. The protein synthesis levels were measured by puromycin assay.

Results: Both Simvastatin and FCCP induce the ATF4 protein levels and its UPR^{mt} target genes but no difference is detected after the co-treatment in cell lines. The relative RNA levels in the tissues tend to be higher after Simvastatin treatment, but there is no statistically significant difference. The protein synthesis levels seem to not be affected.

Conclusion: The repression of the mevalonate pathway by simvastatin treatment induced the ATF4-regulated arm of UPR^{mt} in mammals. Moreover, the total protein synthesis is not affected, besides the activation of ATF4.

P50

Non-classical monocytes in Systemic Lupus Erythematosus (SLE): Molecular and functional characterization

Eirini-Maria Stergioti[1,2], Theodora Manolakou[1,2], Noemin Kapsala[2], Panayotis Verginis[3], Dimitrios Boumpas[1,2], Aggelos Banos[1**]**

[1]Laboratory of Immune Regulation and Tolerance, Autoimmunity and Inflammation, Biomedical Research Foundation of the Academy of Athens, Athens, Greece.
[2]4th Department of Medicine, Attikon University Hospital, Medical School, National and Kapodistrian University of Athens, Athens, Greece.
[3]Laboratory of Immune Regulation and Tolerance, Medical School, University of Crete, Iraklion, Greece.
*** Equal contribution*

Systemic Lupus Erythematosus (SLE) is a complex autoimmune disease characterized by dysfunction and altered homeostasis of monocytes exhibiting an enhanced functional state. In humans, $CD14^{+}$ $CD16^{-}$ (classical) monocytes comprise up ~85% of the circulating monocyte pool, whereas the remaining ~15% consist of $CD14^{+}$ $CD16^{+}$ (intermediate) and $CD14^{lo}$ $CD16^{+}$ (non-classical) monocytes. In SLE patients, non-classical $CD14^{lo}$ $CD16^{+}$ monocytes promote a Th17 phenotype and express TLR7/8 by sensing nucleic acids released from viruses or host suggesting a potential pathogenic role in this disease. To further explore their pathogenic potential in SLE, we performed immunophenotypic characterization of the three monocytic subsets in healthy individuals and SLE patients. We found significant upregulation of CD163 in non-classical monocytes in SLE suggesting increased phagocytic activity of this subset. The phagocytic capacity of non-classical monocytes was further explored by using pHrodo E. coli bioparticles. Non-significant differences were found between steady state and SLE monocytes indicating that non-classical monocytes in the inflammatory environment of SLE may switch into an activated macrophage phenotype promoting production of inflammatory cytokines with diminished ability for phagocytosis. Confocal microscopy revealed that non-classical monocytes exhibited enhanced autophagy in SLE patients with an upregulation of LC3 and downregulation of p62 proteins, indicating a phenotype promoting removal of excessive apoptotic components. *Ex-vivo* studies demonstrated that compared to classical, non-classical monocytes respond better to IFN-α stimulation as shown by the elevated expression levels of the IFN responsive genes (IRF1, IRF4, STAT1, STAT3, STAT4) suggesting priming of SLE monocytes within the SLE milieu and immune memory. On-going experiments involve a) the delineation of the SLE monocytic transcriptional signature by RNA-sequencing for the three monocytic subsets; b) measurement of cytokine production upon TRL7/8 stimulation and c) ChIP experiments combined with transcriptome profiling to determine the epigenetic layer and the transcriptional regulation networks of non classical monocytes.

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High-throughput screening of Hellenic plant extracts for the identification of bioactive natural products with probable anti-ageing properties

Harris Pratsinis[1]*, Aimilia D. Sklirou[2], Maria T. Angelopoulou[1], Aikaterini Argyropoulou[3], Eliza Chaita[3], Vasiliki Ioanna Boka[3], Christina Cheimonidi[2], Katerina Niforou[2], Eleni Mavrogonatou[1], Eleftherios Kalpoutzakis[3], Nektarios Aligiannis[3], Alexios-Leandros Skaltsounis[3], Ioannis P. Trougakos[2], Dimitris Kletsas[1]

[1.] *Laboratory of Cell Proliferation and Ageing, Institute of Biosciences and Applications, NCSR "Demokritos", Athens*

[2.] *Department of Cell Biology and Biophysics, Faculty of Biology, National and Kapodistrian University of Athens*

[3.] *Division of Pharmacognosy and Natural Products Chemistry, Faculty of Pharmacy, National and Kapodistrian University of Athens*

A large number of natural products have been shown to delay cellular senescence of normal human cells or *in vivo* aging of model organisms by regulating age-related signaling pathways. Especially plant extracts may combine anti-aging with photoprotective, antioxidant, anti-inflammatory, and immunomodulatory properties. Greece is gathering the most diverse flora in Mediterranean, being considered one of the global ecological "hot spots". Hellenic flora consists of approximately 6.000 species of higher plants, with approximately 13% of the plant taxa being endemic.

Here, we report an extensive screening among hundreds plant species of the Hellenic flora, in order to identify bioactive natural extracts against age-related proteome instability, and cellular senescence, as well as against more specific targets involved in skin ageing, such as melanogenesis, phototoxicity and extracellular matrix (ECM) catabolism.

More than 440 plant species and subspecies were selected and evaluated. The extracts were initially screened for their antioxidant and anti-melanogenic properties, based on both cell-free (DPPH) and cell-based (DCFH-DA) assays, as well as, on their ability to inhibit tyrosinase activity. The eighteen most potent extracts were selected and were further subjected to cell-based evaluation of their anti-melanogenic activity, assessment of their effects on sirtuin-1 and proteostatic activities, as well as, to assays referring to secreted MMP activity, and protection of dermal cells against oxidative stress-mediated premature senescence and UV-B-induced cytotoxicity. In parallel, the chemical profile of the extracts was analysed with HPTLC and/or UPLC-HRMS.

A variety of extracts were identified that can be of great value e.g. for the cosmetic industry, since they combine antioxidant, photoprotective, anti-melanogenic and anti-ageing properties. Especially, the methanolic extracts of *Sideritis scardica* and *Rosa damascena* could be worthy of further attention, since they showed interesting chemical profiles and promising properties against specific targets involved in skin ageing.

This research has been co-financed by the European Union (European Regional Development Fund – ERDF) and Greek national funds through the Operational Program "Competitiveness and Entrepreneurship" of the National Strategic Reference Framework (NSRF) – Research Funding Program: "Application of state-of-the-art green technology for the development of high added value cosmeceuticals based on the Greek flora-ENGAGE" (11ΣΥΝ_1_420). It was also co-funded by the European Union (ERDF) and Greek national funds through the Operational Program "Competitiveness, Entrepreneurship and Innovation", under the call "STRENGTHENING RESEARCH AND INNOVATION INFRASTRUCTURES" (projects OPENSCREEN-GR and PLANTUP with MIS codes 5002691 and 5002803, respectively).

P52

Isolation of an extract from the soft coral symbiotic microorganism *Salinispora arenicola* exerting cytoprotective and anti-aging effects

Xanthippi P. Louka [1], Aimilia D. Sklirou [1], Géraldine Le Goff [2], Philippe Lopes [2], Eleni-Dimitra Papanagnou [1], Maria S. Manola [1], Yehuda Benayahu [3], Jamal Ouazzani [2] and Ioannis P. Trougakos [1]

[1] *Department of Cell Biology and Biophysics, Faculty of Biology, National and Kapodistrian University of Athens, Athens, Greece*

[2] *CNRS, Institut de Chimie des Substances Naturelles, UPR 2301, Gif-sur-Yvette, France*

[3] *School of Zoology, George S. Wise Faculty of Life Sciences, Tel Aviv University, Ramat Aviv, Tel Aviv, Israel*

Cells have developed a highly integrated system responsible for proteome stability, namely the proteostasis network (PN). As loss of proteostasis is a hallmark of aging and age-related diseases, the activation of PN modules can likely extend healthspan. Here we present data on the bioactivity of an extract (SA223-S2M) purified from the strain *Salinispora arenicola* TM223-S2 that was isolated from the soft coral *Scleronephtya* sp.; this coral was collected at a depth of 65 m from the mesophotic Red Sea ecosystem EAPC (south Eilat, Israel). Treatment of human cells with SA223-S2M activated proteostatic modules, decreased oxidative load and conferred protection against oxidative and genotoxic stress. Furthermore, SA223-S2M enhanced proteasome and lysosomal-cathepsins activities in *Drosophila* flies and exhibited skin protective effects as evident by effective inhibition of the skin aging-related enzymes, elastase and tyrosinase. We suggest that the SA223-S2M extract constitutes a likely promising source for prioritizing molecules with anti-aging properties.

P53

Sumoylation of Exosc10 under hypoxia and its implication in cellular response

Chrysa Filippopoulou, George Simos and Georgia Chachami*
Laboratory of Biochemistry, Faculty of Medicine, University of Thessaly, 41500 Biopolis, Larissa, Greece
**e-mail: ghah@med.uth.gr*

Solid tumors are characterized by reduced oxygen levels (hypoxia) because of high oxygen demands by the proliferating cancer cells and abnormal tumor vascularization. Sumoylation is the covalent attachment of SUMO (Small Ubiquitin-like MOdifier) to target proteins. Recent data support that sumoylation of proteins is required for the activation of the response to hypoxia and the ensuing signaling cascade. By using a SUMO-immunoprecipitation method with quantitative proteomics (SILAC) we have identified proteins that significantly altered their sumoylation status under hypoxia[1]. One such protein is Exosc10 (Exosome subunit 10, or Rrp6), a nucleolar protein and one of the catalytic subunits of the RNA exosome, which has never been previously implicated in the hypoxic response. Mass spectroscopy, immunoprecipitation and immunofluorescence experiments show that hypoxia strongly decreases sumoylation of endogenous and overexpressed Exosc10 and causes a relocation of Exosc10 from the nucleolus to the nucleoplasm. We are currently analysing the hypoxia-regulated sumoylation of Exosc10 using overexpression of sumoylation-deficient Exosc10 mutants and silencing of enzymes of the SUMO machinery. The involvement of Exosc10 and its sumoylation in the adaptation (proliferation, apoptosis, migration) of cancer cells to hypoxia is also being analysed by using CRISPR-Cas9 technology to generate cell lines depleted of Exosc10 or stably expressing its sumoylated and non-sumoylated versions. Our study aims at elucidating sumoylation-dependent regulatory mechanisms that may be critical for tumor growth under low oxygen conditions and which can be targeted by molecular interventions in the context of cancer therapy.

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P54

Identifying cyclic peptides as potential inhibitors of ALS-related protein aggregation using high-content imaging

Maria Tsekrekou[1], **Dafni Delivoria**[1], **Georgios Skretas**[1]
Institute of Chemical Biology, National Hellenic Research Foundation, Athens, Greece

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease with severe socio-economic impact. Aberrant cytoplasmic inclusions of misfolded and aggregated wild type and mutant proteins is a major pathological finding in ALS patients. Despite impressive progress in uncovering the mechanisms underlying pathogenesis, ALS remains an incurable disease and current therapeutic approaches exhibit poor clinical outcomes. Thus, identifying new potential drugs that modify disease outcome is an urgent need. To this end, cyclic peptides due to their high stability, conformational rigidity and low toxicity profile are ideal drug candidates [1]. Within this context, we have established a bacterial platform that allows for screening of up to 200 million cyclic peptides and has been successfully employed to identify candidates with anti-aggregation properties against beta-amyloid (Ab) and Cu/Zn superoxide dismutase (SOD1) [2, 3]. Although cell-based platforms show much lower capacity, they offer a more disease-relevant environment and may provide useful information, e.g. toxicity of the candidate drug. Accordingly, we have employed mammalian cell lines (HEK293T and neuroblastoma-derived SH-SY5Y) over-expressing the ALS-related proteins SOD1(A4V) variant and TAR DNA-binding protein 43 (TDP-43) in the presence of synthetically produced cyclic peptides combined with high-content imaging. Although aggregation is not assayed in cell-based screens, peptides with anti-aggregation activity can be successfully identified by assessing for increased cell viability (live/dead fluorescent staining). Moreover, in the case of SOD1(A4V), fusion of the protein with GFP allows for identifying rescuers of aggregation by monitoring increases in GFP fluorescence. Subsequently, the efficacy and specificity of the candidate inhibitors of aggregation can be further characterized by biochemical and biophysical methods *in vitro*, in cells and eventually validated in pre-clinical models.

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P55

Pharmacogenetic association study of polymorphisms in the canonical and non-canonical NF-kB signaling pathway and response to anti-TNFα therapy in patients with Crohn's disease

Eleana F. Stavrou[1], Fani Chatzopoulou[2,3,4], Charis Antonatos[1], Panagiota Pappa[1], Eutychia Makridou[1], Katerina Trikka[2], Dimitris Chatzidimitriou[4], Konstantinos Oikonomou[5], Andreas Kapsoritakis[5], Petros S Potamianos[5], Konstantinos Karmiris[6], Charalambos Tzathas[7], Ioannis S. Vizirianakis[2,8], Yiannis Vasilopoulos[1*]

[1]Laboratory of Genetics, Department of Biology, University of Patras, Patras, Greece,
[2]Laboratory of Pharmacology, School of Pharmacy, Aristotle University of Thessaloniki, Thessaloniki 54124, Greece,
[3]Labnet Laboratories, Thessaloniki, Greece,
[4]Laboratory of Microbiology, School of Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece,
[5]Gastroenterology Department, University General Hospital of Larissa, Greece,
[6]Gastroenterology Department, "Venizeleio Pananeio" General Hospital of Heraklion, Crete, Greece,
[7]Gastroenterology Department, "Tzaneio" General Hospital of Piraeus, Piraeus, Greece,
*[8]Department of Life and Health Sciences, University of Nicosia, Cyprus, *e-mail: iovasilop@upatras.gr*

Crohn's disease is a gastrointestinal Inflammatory Bowel Disease showing a significantly increasing prevalence worldwide over the years (2.5-3%), mainly in Western world as well as in Greece. Despite the efficacy of anti-TNFα biological agents in inducing remission of Crohn's disease, patients show important heterogeneity (~60-80%) in response to therapy, which is partially due to genetic factors. The aim of our study is to investigate whether functional polymorphisms in the canonical and non-canonical NF-kB signaling pathway could act as potential pharmacogenetic biomarkers of response to anti-TNFα therapy.

Our study included 109 patients diagnosed with Crohn's disease who underwent anti-TNFα therapy for at least 24 months. Disease activity and clinical remission were assessed with Crohn's Disease Activity Index (CDAI). Genotyping of *TLR2* rs3804099 from the canonical and *LTA* rs909253, *TLR4* rs5030728 and *MAP3K14* rs7222094 polymorphisms from the non-canonical signaling pathway was performed with the TaqMan Real-Time PCR method. Statistical analysis and association between genotypes and response to anti-TNFα therapy was performed with Stata 13.1. Patients' average age and duration of disease were 45 and 6.31 years respectively, while 73 (66.7%) patients were positive responders to anti-TNFα therapy. The T allele of *TLR2* rs3804099 showed a statistically significant association with non-response to anti-TNFα agents (P=0.003), even when stratified by drug type (IFX: P=0.032, ADA: P=0.026). Genotypic analysis for the rest of the polymorphisms under study did not reveal any other significant association.

Our results further validate the importance of *TLR2* as a pharmacogenetic biomarker in response to anti-TNFα therapy in patients with Crohn's disease. However, our analysis did not show any association between polymorphisms of the non-canonical NF-kB signaling pathway and response to anti-TNFα therapy. If confirmed in larger populations, our findings suggest that markers of the non-canonical NF-kB signaling pathway could not serve as potential pharmacogenetic markers in anti-TNFα therapy in Crohn's patients.

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P56

McIdas in primary cilium biogenesis

Spyridoula Bournaka[1], Marina Arbi[1], Stavroula Tsaridou[1], Margarita Skamnelou[1], Stavros Taraviras[2], Zoi Lygerou[1*]
[1] Laboratory of Biology, School of Medicine, University of Patras, Rio, Patras, Greece
[2] Laboratory of Physiology, School of Medicine, University of Patras, Rio, Patras, Greece
**e-mail: z_lygerou@yahoo.com*

The primary cilium is a microtubule-based organelle that protrudes from the cell surface upon cell cycle exit and the whole procedure is strictly cell cycle regulated [1]. It is an immotile cilium and many cell signals are transduced through it. A defective primary cilium can be the cause for various diseases, namely ciliopathies. McIdas is a member of the Geminin superfamily that consists of three proteins, Geminin, McIdas and GemC1[2]. Each one of them has an important role not only during the cell cycle but also in cell fate decisions, especially in multiciliated cells. Geminin has also been shown to be implicated in the centrosome cycle, while recent studies in our lab suggest that McIdas controls the centrioles number in cells.

Here, the role of McIdas in the formation of the primary cilium is investigated. The depletion of McIdas leads to significantly reduced number of cells forming a primary cilium. It was then examined at which point of the ciliogenesis McIdas acts. Its depletion does not affect the maturation and the docking of the basal body and its ability to form a primary cilium. Also, neither the ability of the Golgi vesicles to be formed around the basal body is lost. However, the arrange of the actin cytoskeleton of the cell is perturbed upon McIdas depletion and seems to be more similar to that of cycling cells rather than the one of resting cells.

It becomes clear that the members of the Geminin superfamily are involved in an increased number of cellular processes. Understanding their functions will allow their role in the balance between proliferation and differentiation to be elucidated.

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P57

Architecture and druggability of SARS-CoV-2 nsP3c (SARS Unique Domain)

Nikolaos K. Fourkiotis[1], Aikaterini C. Tsika[1], Angelo Gallo[1], Francesca Cantini[2,3], Lucia Banci[2,3], Sridhar Sreeramulu[4], Harald Schwalbe[4] and Georgios A. Spyroulias[1*]

[1] Department of Pharmacy, University of Patras, 26504 Patras, Greece

[2] Magnetic Resonance Center – CERM, University of Florence, Via Luigi Sacconi 6, Sesto Fiorentino, 50019 Florence, Italy

[3] Department of Chemistry, University of Florence, Via della Lastruccia 3, Sesto Fiorentino, 50019 Florence, Italy

[4] Institute for Organic Chemistry and Chemical Biology, Johann Wolfgang Goethe-University Frankfurt, Max-von-Laue-Str. 7, 60438 Frankfurt, Germany

Among the protein domains that are common in several Betacoronaviruses (β-CoVs) are, the so-called, SARS Unique Domains (SUDs), first identified in SARS-CoV. In SARS-CoV-2, the polypeptide non-structural protein 3 (nsP3), namely nsP3c, includes the SUDs domains. It consists of three distinct domains: a) SUD-N that is located at the N-terminal of SUD; b) SUD-M (middle domain); (c) SUD-C that is the smallest of the three. SUD-N and SUD-M retain a macro-like fold, lacking the enzymatic activity of macro domains, while SUD-C exhibits a frataxin-like motif. Although, these domains lack the typical macro features to bind ADP-ribose and other NAD+ metabolites, SUD-N and SUD-M exhibit affinity against oligonucleotides rich in guanine notably for those forming secondary structures like G-quadruplexes and SUD-C might play a role in tuning the selectivity of SUDs. This specific affinity suggests that they might play a role in the virus replication-transcription complex (RTC). In addition, a recent study demonstrated that SUD-MC interacts with specific cellular components and significantly up-regulating the expression of chemokines, resulting in pulmonary inflammation. Taken together their role and their interactions, SUDs might be exploited as drug targets.

Herein we present the NMR characterization and interaction of SUDs, that may be of great interest for the detailed functional characterization of the viral components and/or the discovery or the identification of new lead compounds that bind to these proteins in the quest for new antiviral drugs.[I, II]

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P58

Studying the role of non-RING elements of Arkadia in the E2-E3 interaction

Maria Birkou[1], Georgia N. Delegkou[1], Konstantinos D. Marousis[1], Vasso Episkopou[2], Georgios A. Spyroulias[1*]
[1]Department of Pharmacy, University of Patras, Patra, Greece
[2]School of Medicine, Imperial College, London, United Kingdom

Ubiquitination is a post-translational modification in which ubiquitin is transferred to a target protein through the concerned actions of three enzymes, the E1 activator enzyme, the E2 conjugating enzyme and the E3 ubiquitin ligase. The E3 enzymes are the key components of the process, as they are responsible for substrate recognition and poly-ubiquitination. Biochemical and structural studies have shown that RING domains bind on E2~Ub and stabilize the active "closed" conformation of E2~Ub conjugate. Although RING domains are sufficient to stabilize the E2~Ub conjugates in the "closed" conformation, additional regions outside the RING domain contribute to maintain this state and enhance the efficiency of ubiquitin transfer[1].
Arkadia is an E3 ubiquitin ligase that positively regulates the TGF-β pathway by targeting its' negative regulators Smad7, c-Ski and SnoN for ubiquitin-dependent degradation, through its C-terminus RING domain[2]. Biochemical studies revealed the significant role of the conserved (among species) NRG and TIER segments, which are preceding the RING domain of Arkadia, in the substrate recognition and ubiquitination[3]. In this work the role of these segments in the E2-E3 interaction is studied, using its physiological partner UbcH5b E2 enzyme. More specifically, NMR interaction studies and oxy-ester hydrolysis assays of UbcH5b~Ub conjugate were carried out using various Arkadia's constructs, to determine the role of these segments in Arkadia-dependent ubiquitination process.

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The biogeography of Greek table olives varieties assessed by amplicon-based metagenomics analysis

Lena Pagiati*, Maria Kazou, Effie Tsakalidou
Laboratory of Dairy Research, Department of Food Science and Human Nutrition, Agricultural University of Athens, Greece
* lenapagiati@aua.gr

In recent years and in the field of food research, the terms microbial terroir and biogeography have been introduced, identifying patterns of microbial diversity across defined spatial or temporal scales that can be used as a food authentication tool. The present study focuses on the microbial fingerprinting of the most important Greek olive varieties using the amplicon-based metagenomics analysis. Towards this, 61 samples from 38 olive varieties were collected at the final stage of ripening from 13 well-spread geographical regions in Greece. For the metagenomics analysis, total DNA was extracted from the olive samples and the 16S rRNA gene and the ITS DNA region were sequenced and analyzed using bioinformatics tools for the identification of bacterial and yeast/fungi diversity, respectively. Furthermore, principal component analysis (PCA) was performed for data clustering based on the average microbial composition of all samples from each region. Bacterial microbiota revealed that each region had a unique microbial fingerprint. However, this was not the case for the yeast/fungi microbiota, since 10 out of the 13 regions were grouped together mainly due to the dominance of the genus *Aureobasidium*. A second cluster was formed for the islands Crete and Rhodes, both located in the Southeast Aegean sea. Finally, the Agrinio region did not cluster with any of the rest showing a completely different microbial fingerprinting. The present study is part of a bigger project, the first of its kind in Greece, focusing on the employment of metagenomics approach in order to unravel the biogeography of the Greek table olives varieties and the impact of the indigenous microbiota on the quality and safety of the fermented table olives.

Keywords Amplicon-based metagenomics analysis, bacteria, microbial biogeography, olive microbiota, yeasts/fungi

P60

Immunoinformatics-aided design and immunogenicity evaluation of a multi-epitope vaccine against visceral leishmaniasis

Maritsa Margaroni[1], Maria Agallou[1], Evgenia Tsanaktsidou[2], Olga Kammona[2], Costas Kiparissides[2], Evdokia Karagouni[1]

[1] *Immunology of Infectious Diseases Laboratory, Department of Microbiology, Hellenic Pasteur Institute, Athens, Greece*

[2]*Chemical Process and Energy Resources Institute, Centre for Research and Technology Hellas, Thessaloniki, Greece*

An ideal vaccine against leishmaniasis should be effective against all different forms of disease and also target both stages, promastigote and amastigote, of the parasite life cycle. Recent advances in reverse vaccinology could be a promising approach for designing an efficient vaccine against leishmaniasis. In the present study, comparison with the VACCEED tool of *L. infantum*, *L. major* and *L. braziliensis* parasites' whole proteome revealed 232 proteins with >80% homology among the three species. These proteins were further analyzed for the identification of Helper (HTL) and Cytotoxic (CTL) T lymphocytes epitopes by using different immunoinformatics tools, such as NetMHCpan, SYFPEITHI and IEDB algorithms. The highly-scored epitopes were selected and were fused in tandem to construct a multi-epitope protein (L.chimera2) consisting of 407 aminoacids and molecular weight 45.500,57 Da. Computational analysis revealed that L.chimera2 was antigenic and non-allergenic with low homology against human or murine aminoacid sequences. PSIPRED and i-TASSER algorithms were used to predict its secondary and tertiary structure, whereas refinement and validation of the predicted model was also conducted. Several linear and conformational B-cell epitopes were determined within L.chimera2, whereas molecular docking analysis with MHCI and MHCII molecules was performed. Finally, C-ImmSim server predicted in silico an immunogenic profile for the protein. Thus, in order to confirm the in silico prediction and to evaluate the immunogenicity of L.chimera2 in vivo, the protein was loaded to, FDA approved, PLGA nanoparticles with MPLA as adjuvant. Intramuscular injection of BALB/c mice with the nanoparticulate vaccine induced antigen-specific spleen cell proliferation as well as the development of $CD4^+$ T memory cells, suggesting L.chimera2 as a worth-studying vaccine candidate against leishmaniasis.

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Epigenetic coupling of transcription factor CXXC5 regulates stemness genes in glioblastoma

Kalliopi Tzavlaki[1], Mahsa Shahidi Dadras[2], Artur Mezheyeuski[2], Anita Morén[1], Carl-Henrik Heldin[1], Laia Caja[1] and Aristidis Moustakas[1,*]

[1] Department of Medical Biochemistry and Microbiology, Science for Life Laboratory, Box 582, Biomedical Center, Uppsala University, SE-75123 Uppsala, Sweden.

[2] Department of Immunology, Genetics and Pathology, Box 256, Rudbeck Laboratory, Uppsala University, SE-75105 Uppsala, Sweden.

Glioblastoma (GBM) is a brain malignancy characterized by high proliferation and invasiveness. Signaling by members of the transforming growth factor β (TGFβ) family controls fate decisions between self-renewal and differentiation in GBM stem-like cells. TGF-β signaling promotes stemness whereas bone morphogenetic protein (BMP) signaling promotes differentiation in GBM. Identifying new target genes of TGFβ/BMP signaling in GBM cells can explain how TGFβ and BMP pathways regulate fate choices between stemness and differentiation in human GBM stem-like cells. Transcriptomic analysis revealed that TGFβ and BMP regulate CXXC5 expression, a CXXC-type zinc finger transcription factor, which binds to CpG islands. Multiparametric immunohistochemistry in human GBM tissue demonstrated CXXC5 enrichment in cells expressing the stemness proteins SOX2 and NESTIN. Accordingly, silencing of endogenous CXXC5 reduced glioma-sphere forming capacity, and regulated either positively or negatively, expression of several genes, including genes whose expression can be regulated by TGFβ and BMP signaling. To obtain mechanistic insight, mass-spectrometric analysis of proteins interacting with endogenous CXXC5 identified chromatin remodelers including the KMT2C methyltransferase, suggesting epigenetic regulation of gene expression by CXXC5. Functionally, CXXC5 and KMT2C association, via histone methylation, regulates the *leukemia inhibitory factor* and *NESTIN* genes that control GBM stemness. By controlling the recruitment of KMT2C/COMPASS and SUZ12/PRC2 to GBM stemness genes, CXXC5 contributes to their "bivalent" chromatin, allowing tumor cell fate to wobble between stemness and differentiation. Low CXXC5 expression shifts stemness genes to a repressed state, leading to reduced self-renewal and better prognosis. TGFβ and BMP regulate CXXC5 expression, thereby modulating the "bivalent" status of tumor cell chromatin. We propose that CXXC5 is one of the key chromatin regulators that explain why growth factors such as TGFβ and BMP fail to generate GBM cells that are respectively purely stem-like or fully differentiated, as proposed by studies in diverse cohorts of GBM patients.

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The GPCR lipid/androgen receptor OXER1 is up-regulated by its ligand 5-oxo-ETE and has a prominent place in human cancer cell migration

Konstantina Kalyvianaki, Irene Drosou, George Notas, Elias Castanas, Marilena Kampa*
Laboratory of Experimental Endocrinology, University of Crete, School of Medicine, Heraklion, Greece
**e-mail: kampam@uoc.gr*

Background: 5-Oxo-6,8,11,14-eicosatetraenoic acid (5-oxo-ETE) is a potent eosinophil chemoattractant and activator that is synthesized not only in inflammatory but also in epithelial cells. The receptor for 5-oxo-ETE, OXER1 is a recently identified receptor implicated in chemoattraction of circulating mononuclear cells, Ca^{2+} surge in neutrophils, inflammation and cancer. Recently, we have shown that OXER1 is also a membrane androgen receptor in various cancer tissues. It was reported that the presence of OXER1 in leucocytes and the production and release of 5-oxo-ETE by wounded tissues is a wound sensing mechanism, leading to lymphocyte attraction. In view of the similarity of hallmarks of cancer and wound healing, the purpose of this study is to clarify whether OXER1 and its endogenous ligand could hold an important part in the activation/migration phase of healing.
Materials and methods: We have explored the role of OXER1 and 5-oxo-ETE, in the control of cell migration of human cancer epithelial cells (DU-145, T47D and Hep3B), mimicking the activation/migration phase of healing. OXER1 expression was detected by qPCR, FACS and Western blotting analysis. Knock-down of OXER1, incubation with 5-oxo-ETE, and inhibition of 5-oxo-ETE production were examined on cell migration (wound-healing assay) and actin cytoskeleton (visualization with rhodamine-labelled phalloidin).
Results: OXER1 is up-regulated only at the leading edge of the wound and its expression is up-regulated by its ligand 5-oxo-ETE, in a time-related manner. Knock-down of OXER1 or inhibition of 5-oxo-ETE synthesis led to decreased migration of cells and a prolongation of healing, in culture prostate cancer cell monolayers, with a substantial modification of actin cytoskeleton and a decreased filopodia formation. Inhibition of cell migration is a phenomenon mediated by Gβγ OXER1 mediated actions.
Conclusion: These results provide a novel mechanism of OXER1 implication in cancer progression and might be of value for the design of novel OXER1-targeted therapeutic approaches.

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Development of open-source tools for automatic analysis of DNA damage repair in high-content screens

Ourania Preza[1], Nibal Badra Fajardo[1], Stavros Taraviras[2], Zoi Lygerou[1*]
[1]Laboratory of General Biology, School of Medicine, University of Patras, Greece,
[2]Laboratory of Physiology, School of Medicine, University of Patras, Greece
**e-mail: lygerou@upatras.gr*

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. Therefore, developing tools under a user-friendly environment for automatic analysis is crucial. In our group, we have developed custom-made pipelines for automatic analysis of high-content 2D or 3D images. We show here 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, and shedding light into the molecular mechanisms underlying these events could help unveiling novel targets for cancer therapy.

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Novel therapeutics for fatty acid oxidation disorders

Margarita Tenopoulou[1], Harry Ischiropoulos[2] and Paschalis-Thomas Doulias[1,3]*
[1]Laboratory of Biochemistry, School of Chemistry, University of Ioannina, Ioannina 45221
[2]Children's Hospital of Philadelphia Research Institute and Departments of Pediatrics, The Raymond and Ruth Perelman School of Medicine at the University of Pennsylvania, PA, 19104
[3]Institute of Biosciences, University Research Center of Ioannina (U.R.C.I.), Ioannina, Greece

Long-chain fatty acid oxidation disorders (LC-FAODs) are a group of rare, life-threatening, genetic disorders caused by inherited defects in transport proteins or metabolic enzymes in the mitochondrial long-chain fatty acid β-oxidation pathway, preventing the conversion of long-chain fatty acids into energy. Currently, there is an unmet need to develop and test corrective therapeutics for LC-FAODs.
Herein, we provide evidence for the therapeutic efficacy of a hybrid molecule named 2-mononitrate-1,3-diheptanoin (MNDH). We reason that MNDH retains the anaplerotic function of triheptanoin (TH) (1) while it furnishes bioactive nitric oxide (NO) equivalents inside the mitochondria to augment enzymatic and transporter activities via selective S-nitrosylation. The scientific premise for this novel molecule is based on our previous observations that NO through selective S-nitrosylation of enzymes and transporters participating in LC-FAO confer an increase in function (2,3). The coordinated gain-of-function is manifested by an augmented flux for palmitate oxidation and a restoration of a normal acylcarnitine profile.
To begin testing MNDH, we exposed non-disease fibroblasts to MNDH and showed a time and concertation dependent increase in the levels of protein S-nitrosocysteine indicating that metabolism of MNDH generates NO equivalents. A dose dependent increase of FAO flux was also documented. For one of the enzymes, very long chain acyl-CoA dehydrogenase (VLCAD) we also demonstrated selective S-nitrosylation that corresponded with an increase in enzymatic activity.
The efficacy of MNDH was tested in fibroblasts harboring VLCAD mutations. Treatment with MNDH but not TH restored the VLCAD specific activity and FAO flux concomitant with the S-nitrosylation of VLCAD.
These data provide the first evidence for the efficacy of delivering bioactive NO to prevent biochemical abnormalities caused by VLCAD deficiency. Current studies are investigating the efficacy of MNDH to restore metabolic and phenotypic deficiencies in the setting of VLCAD, carnitine palmitoyltransferase 2, CPT2 and mitochondrial trifunctional protein deficiency.

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Metabolomic analysis of interactions between plants and phytopathogenic bacteria

Veronica S. Giourieva[1], Lefteris Andriotis[1], Spyros Didos[2], Foteini Trikka[2], Anagnostis Argyriou[2], Dimitrios A. Kyriakidis[1], Rigini M. Papi[1]*

[1] *Department of Chemistry, Aristotle University of Thessaloniki, Greece*

[2] *Centre for Research and Technology Hellas, Thessaloniki, Greece*

Plant diseases account for a significant portion of crop losses worldwide, in addition to those caused by abiotic stresses such as drought or saline soils[1,2]. Crop losses to viruses alone can be up to 82% in banana and 100% in cocoa[3]. Bacterial, fungal, viral, and parasitic nematode diseases resulted in an estimated $26 billion loss from 2010 through 2014 in the United States soybean crop alone[2,4]. Metabolomics can provide a snapshot of plant metabolism during development and in response to a wide range of biotic and abiotic stimuli, including environmental or nutritional stresses[2]. The purpose of this study was the development of a simple, low-cost and accurate method for the rapid identification of plant infections. Thus, the plant-phytopathogen systems *Arabidopsis thaliana* – *Pseudomonas syringae* pv. *maculicola* ES4326 and *A. thaliana-Xanthomonas campestris* pv. *campestris* were evaluated. Untargeted metabolomics was applied by LC-MS/MS to bacterium-infiltrated leaf in comparison to distal and mock leaves. In addition, the chemical profile was produced by the Fourier transform Infrared (FT-IR). For the *A. thaliana-P. syringae* pathosystem 83 and 217 compounds at 4.5 hpi (hours post infiltration) and 24 hpi, respectively, were identified out of which 11 were related to *A. thaliana* response to phytopathogens/or response to wound, such as salicylic acid and abscisic acid. FT-IR analysis showed differentiation of the dynamic changes of the spectra between infected and uninfected leaves. For *A. thaliana-X. campestris* LC-MS/MS detected more than 70 different compounds in each timepoint out of which 8 compounds were related to *A. thaliana* defense against the pathogen, such as epi-jasmonic acid and p-coumaroylagmatine. However, FT-IR did not retrieve significant band changes. To conclude, combination of FT-IR and LC-MS/MS is a promising tool for the rapid detection of plant infection onset.

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P66

Autophagy activation can partially rescue proteasome dysfunction-mediated cardiac toxicity

Eleni-Dimitra Papanagnou[1]*, Sentiljana Gumeni[1], Aimilia D. Sklirou[1], Alexandra Rafeletou[1], Evangelos Terpos[2], Kleoniki Keklikoglou[3,4], Efstathios Kastritis[2], Kimon Stamatelopoulos[2], Gerasimos P. Sykiotis[5], Meletios A. Dimopoulos[2], Ioannis P. Trougakos[1]

[1]Department of Cell Biology and Biophysics, Faculty of Biology, National and Kapodistrian University of Athens, 15784 Athens, Greece

[2]Department of Clinical Therapeutics, School of Medicine, National and Kapodistrian University of Athens, 11528 Athens, Greece

[3]Institute of Marine Biology, Biotechnology and Aquaculture, Hellenic Centre for Marine Research (HCMR), 71003 Heraklion, Crete, Greece

[4]Biology Department, University of Crete, Heraklion, Crete, 70013, Greece

[5]Service of Endocrinology, Diabetology and Metabolism, Lausanne University Hospital, CH-1011 Lausanne, Switzerland

The ubiquitin-proteasome pathway and its functional interplay with other proteostatic and/or mitostatic modules are crucial for cell viability, especially in post-mitotic cells like cardiomyocytes, which are constantly exposed to proteotoxic, metabolic and mechanical stress. Consistently, treatment of multiple myeloma patients with therapeutic proteasome inhibitors may induce cardiac failure; yet the effects promoted by heart-targeted proteasome dysfunction are not completely understood. We report here that heart-specific proteasome knockdown in the fly experimental model results in increased proteome instability and defective mitostasis, leading to disrupted cardiac activity, systemic toxicity, and reduced longevity. These phenotypes were partially rescued by either heart targeted- or by dietary restriction-mediated activation of autophagy. Supportively, activation of autophagy by Rapamycin or Metformin administration in flies treated with proteasome inhibitors reduced proteome instability; partially restored mitochondrial function, mitigated cardiotoxicity, and improved flies' longevity. Our findings suggest that autophagic inducers represent a novel promising intervention against proteasome inhibitors-induced cardiovascular complications.

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Neuroproteomic analysis of mitochondrial function: Novel tool in the discovery of Alzheimer's early-stage diagnosis biomarkers

Danai Psaradelli [1,2,4], Anastasia Chatzisavvidou[1], Konstantina Psatha[1,2,3], Michalis Aivaliotis*[1,2,3]
[1]*Laboratory of Biological Chemistry, School of Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece*
[2]*Functional Proteomics and Systems Biology laboratory (FunPATh), Center for Interdisciplinary Research and Innovation (CIRI-AUTH), Thessaloniki, Greece*
[3]*Institute of Molecular Biology and Biotechnology (IMBB) - Foundation for Research and Technology (FORTH), Heraklion, Greece*
[4]*Department of Biology, Davidson College, North Carolina, U.S.A.*
**email: aivaliotis@auth.gr*

AIM: Alzheimer's is an untreatable, progressive neurodegenerative disease caused by amyloid Aβ42 and tau protein accumulation in the brain area. Disease diagnosis is limited to the detection of APP, PSEN1, PSEN2, and E4 proteins, and the fulfillment of designated neurophysiological criteria. Considering the disease's aggressive nature and the lack of precision diagnostic methods, the discovery of diagnostic biomarkers with high sensitivity and specificity is crucial. Systematic study of CNS's proteome using high resolution mass spectroscopy will result in the discovery of biomarkers leading to a more meaningful comprehension of the disease's molecular basis. Mitochondrial dysfunction [3] may be a very promising target since it significantly affects cell function and protein expression.
MATERIALS AND METHODS: The present research project emerged from reviewing the available bibliography on the Alzheimer's disease on the databases PubMed, Science Direct, and NCBI published in the time frame 2016-2021. Bibliography was collected using specific, pre-defined key words and the Boolean logic. The keywords were: ("Alzheimer's" OR "Alzheimer's Disease") AND (("Mitophagy") OR (("Mitochondria") AND ("PINK1"))). Screening and data extraction followed the PRISMA statement guidelines (PRISMA Flow Diagram, 2020). From the 1951 publications, 321 were retrieved after the first screening.
RESULTS: The aforementioned methodology led to the emergence of novel protein targets such as PINK1[1], protein associated with dysfunctional mitochondria's mitophagy, SIRT1[4], and Drp1[2]. These proteins demonstrate great potential as preclinical stage Alzheimer's biomarkers.
CONCLUSION: Dysfunctional mitochondria accumulation and the downregulation of proteins related to mitochondrial function are processes detected in the early stages of the disease and should be studied as potential early diagnosis biomarkers and pharmacological targets. Application of a neuroproteomics approach can be a unique tool in linking potential risk factor mitochondrial proteins to Alzheimer's early-stage clinical diagnosis, supporting treatment perspectives.

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P68

Homologous recombination competes with alternative DNA repair pathways to induce telomerase independent lengthening of telomeres in *C. elegans*

Fivos Borbolis[1], Konstantinos Giatras[1,2], Sarantis Gagos[1]*, Popi Syntichaki[1]*
[1]*Biomedical Research Foundation of the Academy of Athens, Greece*
[2]*National and Kapodistrian University of Athens, Greece*

The end replication problem poses a serious challenge that all diving eukaryotic cells have to face in order to maintain the length of their chromosomal extremities (telomeres). Shortening of telomeres beyond a critical limit induces DNA damage responses and leads to senescence, thereby limiting the number of cell divisions. Moreover, short telomeres have been linked to increased frequency of chromosome non-disjunctions that lead to aneuploidy during meiotic divisions. Telomere lengthening is mainly mediated by the reverse transcriptase telomerase. However, in the absence of telomerase, an alternative telomere elongation mechanism (ALT) has been reported. From yeast to humans this recombination-based mechanism shows features of break induced replication (BIR) DNA repair. However, the details about its interspecies variations and the mechanistic context that drives its implementation are still elusive. The nematode *C. elegans* can live for multiple generations without a functional telomerase enzyme, providing excellent grounds to study ALT. Nonetheless, long-term transgenerational survival necessitates the existence of large populations, suggesting that stochastic events beyond ALT activation may be involved. Here we apply a targeted RNAi mini-screening approach on a telomerase null mutant worm strain (*trt-1*$^{-/-}$), to identify key factors that mediate ALT implementation and investigate their impact on transgenerational fertility maintenance, and aneuploidy frequency. Our work provides evidence that RAD51-dependent homologous recombination is essential even for short-term maintenance of small *trt-1*$^{-/-}$ populations and affect the frequency of chromosome non-disjunction events, while the function of known BIR associated factors such as RAD52 seems to be dispensable. Conversely, we find that factors of the Fanconi complex, involved in alternative DNA repair pathways, impair transgenerational survival and promote aneuploidy. Our results suggest a competing relationship between homologous recombination and alternative DNA repair pathways in telomeric regions that ultimately regulates ALT induction and execution.

P69

TGFβ differentiates pro-stemness from pro-invasive phenotypes during cancer cell EMT

Yutaro Tsubakihara[1-2], Yae Ohata[1], Yukari Okita[2], Shady Younis[1-3], Jens Eriksson[1], Mikael E. Sellin[1], Jiang Ren[4], Peter ten Dijke[4], Kohei Miyazono[5], Atsuhiko Hikita[6], Takeshi Imamura[7], Mitsuyasu Kato[2], Carl-Henrik Heldin[1] and Aristidis Moustakas[1] *

[1] *Dept. Medical Biochemistry and Microbiology, Uppsala University, Sweden.*
[2] *Dept. Experimental Pathology, University of Tsukuba, Japan.*
[3] *Dept. Medicine, Stanford University, USA.*
[4] *Dept. Cell & Chemical Biology, Leiden University Medical Center, The Netherlands.*
[5] *Dept. Molecular Pathology, The University of Tokyo, Japan.*
[6] *Div. Tissue Engineering, The University of Tokyo Hospital, Japan.*
[7] *Dept. Molecular Medicine for Pathogenesis, Ehime University, Japan*

The epithelial-mesenchymal transition (EMT) fuels embryonic tissue development and adult pathogenesis in the context of fibrosis or cancer metastasis. EMT is induced by transforming growth factor β (TGFβ), and molecularly links to processes of cancer cell stemness and invasiveness. Mesenchymal cells can revert to epithelial cells through the inverse process called mesenchymal-epithelial transition (MET). It remains unclear as to how TGFβ differentiates cancer stemness or invasiveness via EMT. Aiming at identifying contexts that could reveal differences in phenotypic responses to TGFβ, we established a breast cancer fluorescent cell model that is based on its responsiveness to TGFβ. The *E-cadherin* promoter, as a fluorescent marker driver, best phenocopied the majority of EMT and MET features. TGFβ promoted 3D oncosphere formation with low-RFP content, suggesting enrichment of epithelia-mesenchymal cells (partial EMT) in the oncosphere. Under 3D context, autocrine TGFβ supported dynamic generation of non-fluorescent mesenchymal cells that initiated explorative migration in the surrounding space. The autocrine TGFβ action was verified by measuring its secretion extracellularly and by blocking its biological action using the potent TGFβ type I receptor inhibitor LY2157299. In contrast, prolonged exogenous TGFβ stimulation failed to support motility from oncospheres but instead enhanced oncosphere growth. After EMT, TGFβ also induced extracellular vesicle secretion, transporting pro-EMT signals onto recipient cells via a rich transcriptomic population, assessed by sequencing of the vesicular cargo RNAs. Among, these cargo mRNAs, and after cloning 16 of them, we identified novel regulators of TGFβ signaling. Orthotopic mammary transplantation experiments of oncospheres in mice, revealed that prolonged exogenous TGFβ treatment promoted tumor-initiating capacity while it failed to support tumor intravasation and lung metastasis. Thus, pro-stemness or pro-invasive phenotypes by TGFβ are differentiated based on the multicellular architectural context into which the EMT takes place.

P70

Inhibition of Akt1/mTOR pathway promotes autophagy and clearance of Group B Streptococcus in alveolar epithelial cells

Ioanna Pantazi[1,3], Iosif Papafragkos[2], Ourania Kolliniati[1], Eleni Vergadi[3], Christos Tsatsanis[1*]
[1]*Laboratory of Clinical Chemistry, Medical School, University of Crete, Heraklion 71003, Greece*
[2]*Laboratory of Immune Regulation & Resistance, Medical School, University of Crete, Heraklion 71003, Greece*
[3]*Department of Pediatrics, Medical School, University of Crete, Heraklion 71003, Greece*

Group Beta Streptococcus is a Gram-positive encapsulated bacterium that is usually harmless in healthy adults, but constitutes a serious pathogen for vulnerable groups such as neonates and young infants, causing pneumonia, sepsis, and meningitis[1]. Innate immunity is the main defense mechanism of newborns, since their adaptive immune system is neither mature nor adequately trained. Specifically, in the case of GBS infection neonates might be benefited by an enhanced first line of defense offered by the alveolar epithelial cells, being the initial site of GBS entry to the circulation. Therefore, in the present study we sought to investigate whether we can enhance the antibacterial responses of alveolar epithelial cells, in order to hamper GBS blood invasion and improve survival from sepsis. Based on previous studies, demonstrating an enhanced bactericidal capacity of Akt1 deficient murine macrophages[2,3], we decided to focus on Akt1 inhibition, using the Akt inhibitor MK2206 on the alveolar epithelial cell line A549. Our results revealed enhanced bacterial clearance, higher ROS production and increased inflammatory responses in MK2206 treated cells, indicating that Akt1 inhibition can enhance the bactericidal capacity of alveolar epithelial cells. Targeting critical autophagy components, such as ATG5 and LC3II, we demonstrated that this enhanced bactericidal capacity is mediated through induction of autophagy, resulting from a dysregulated PI3K/Akt/mTOR pathway. The enhanced antibacterial properties gained by Akt1 inhibition were further proven *in vivo*, with MK2206 administration on C57BL/6 neonatal pups. Thus, our study supports that inhibition of Akt1 kinase constitutes a potent therapeutic target, which can enhance the antibacterial responses of the lung epithelium, as a preventive measure for GBS invasion and dissemination.

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P71

Effect of belimumab therapy on atheroprotective properties of HDL in systemic lupus erythematosus

Anastasia-Georgia Dedemadi[1], Christina Gkolfinopoulou[1], Dimitra Nikoleri[2,3], Myrto Nikoloudaki[2], Prodromos Sidiropoulos[2,3], George Bertsias[2,3] and Angeliki Chroni[1]*

[1]Institute of Biosciences and Applications, National Centre for Scientific Research "Demokritos", Agia Paraskevi, Athens, Greece

[2]Laboratory of Rheumatology, Autoimmunity and Inflammation, University of Crete Medical School, Heraklion, Greece

[3]Institute of Molecular Biology and Biotechnology, FORTH, Heraklion, Greece

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease, which is characterized of excessive inflammation and is associated with increased risk of cardiovascular disease (CVD). High levels of pro-atherogenic low-density lipoprotein (LDL) and low levels of high-density lipoprotein (HDL) are important risk factors for atherosclerotic cardiovascular complications in SLE patients. However, these traditional risk factors have failed to explain the increased risk of CVD in these patients. Furthermore, HDL functionality, that has been shown to be more important than its concentration for atheroprotection and CVD risk, is impaired during chronic inflammatory conditions such as SLE. The aim of this study was to investigate the effect of belimumab (a B-cell-activating factor (BAFF) or B-lymphocyte stimulator (BlyS) inhibitor) treatment on atheroprotective properties of HDL in SLE. We measured HDL antioxidant capacity, HDL-associated paraoxonase-1 (PON1) activities and HDL-associated myeloperoxidase (MPO) and serum amyloid A (SAA) levels in 35 samples of SLE patients before and following treatment with belimumab for 6 months. After 6 months of treatment, the HDL antioxidant capacity was improved, accompanied by increases in HDL-associated PON1 paraoxonase and arylesterase activities. HDL-associated MPO and SAA levels remained unchanged. Our findings show that the HDL atheroprotective function can be improved with the inhibition of BAFF/BLyS in patients with SLE possibly affecting atherosclerosis development in SLE.

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P72

Deep Proteome Profiling of Membrane Cargo Trafficking Proteins in *Aspergillus nidulans* Under N Source Derepressing Conditions

Xenia Georgiou[1,2], Sofia Dimou[1], Eleana Sarantidi[2], George Diallinas[1]*, Athanasios K. Anagnostopoulos[2] *

[1.] *Department of Biology, National and Kapodistrian University of Athens, Athens, Greece*

[2.] *Division of Biotechnology, Center of Systems Biology, Biomedical Research Foundation of the Academy of Athens (BRFAA), Athens, Greece*

Background/Aim: Solute and ion transporters are proteins essential for nutrition, detoxification, signaling and homeostasis of the living cell. During their trafficking transporters interact dynamically and transiently with multiple proteins, whilst their exact route from the endoplasmic reticulum to their final membrane destination has yet to be defined. The aim of the study was to elucidate trafficking mechanisms correlated to transporter subcellular translocation by in depth analyzing *Aspergillus nidulans* proteins, as well as delivering a universal protein extraction and identification protocol for the model organism.

Material and Methods: *A.nidulans* strains were grown in conditions favoring yield of proteins related to nitrogen source starvation, in order to elucidate the trafficking process of transporters of interest (nucleobase transporters). A transformed strain was used, expressing a transporter fused to a robust biotin ligase, thus enabling proximity-dependent biotinylation to be employed. To enhance extraction efficacy, proteins were treated with a variety of different denaturizing buffers (e.g. SDS, Chaps, Urea). Proteins were digested to peptides by trypsin and peptide separation was performed in a nanoHPLC system. Mass spectra were collected in an Orbitrap Elite mass spectrometer and the resulting data were processed using Proteome Discoverer. MS2 spectra were searched with the SEQUEST engine against the A. nidulans protein database (UniProtKB).

Results: Our approach resulted in identification of 5,690 proteins (1), constituting the largest protein dataset ever identified in *Aspergilli*. Our analysis unambiguously detected the majority of proteins necessary for key cellular processes, including proteins of paramount importance for cargo membrane trafficking and turnover (e.g. sec23, sec24, ARFs, Rabs). Specifically, 2%, 4% and 6% of the proteome is associated with the fundamental processes of macromolecule localization, vesicle-mediated transport and transmembrane transport, respectively. This result highlights the plethora of proteins involved in the proper localization of different molecules, that in many cases remains to be determined.

Discussion: Our study delivered the definite proteome of *A.nidulans* under N-source depressing conditions, whilst clarifying trafficking mechanisms of key membrane molecules. A universal protocol for protein extraction and identification applicable to all aspergilli was standardized. Our approach and data on *A.nidulans* paves the way for further proteomic studies using the proximity biotinylation labelling set up.

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P73

STAT5 target gene networks in leukemia

Eirini Sofia Fasouli and Eleni Katsantoni*
Basic Research Center, Biomedical Research Foundation, Academy of Athens, Greece
*ekatsantoni@bioacademy.gr

Signal transducer and activator of transcription 5 (STAT5) is an important regulator involved in several cellular processes including cell proliferation and survival. Abnormal STAT5 signaling has been implicated in various solid and hematologic malignancies. Myelodysplastic Syndrome (MDS) is a heterogeneous group of clonal hematologic disorders, characterized 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), which is an aggressive hematologic malignancy, characterized by the accumulation of immature myeloblasts in the bone marrow and the peripheral blood. Constitutive activation of STAT5 has been linked to a plethora of hematopoietic malignancies including AML. To elucidate the role of STAT5 in MDS to AML transformation, this project aims to identify changes in STAT5 target gene networks. To this end STAT5A or STAT5B knock-downs have been generated in MDS and AML cell lines using sh-RNAs through lenti-viral delivery and 3'-mRNA-sequencing has been performed. Differentially expressed genes between knock-downs and controls have been determined, together with changes between STAT5A and STAT5B target gene networks in each cell line. Differences in target gene networks were also defined between the MDS and the AML cell lines for both STAT5A and STAT5B factors. Gene ontology analysis confirmed alterations in various cellular pathways and biological functions. Our data provide a map of STAT5A and STAT5B target genes in MDS and AML cell lines, which will be validated in human hematopoietic stem and progenitor cells at the bulk and single-cell level. It is expected that the identified target gene networks will define the differential role of STAT5A and STAT5B in leukemic transformation and will provide new targets for the therapeutic management of leukemia.

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Carnosic acid and Carnosol display anti-oxidant and protective anti-prion properties in *in vitro* and *cell-free* models of prion diseases

Konstantinos Xanthopoulos[1], Eirini Kanata[1], Spyros Pettas[2], Korina Karagianni[2], Aikaterini Karsanidou[1], Nikolaos Bekas[2], Elisavet Lioulia[2], Katrin Thüne[3], Matthias Schmitz[3], Inga Zerr[3], Franck Llorens[3,4,5], Ioannis Tsamesidis[6,7], Evgenia Lymperaki[7], Theodoros Sklaviadis[1], Dimitra Dafou[2]

[1] Laboratory of Pharmacology, School of Pharmacy, Aristotle University of Thessaloniki, Thessaloniki, Greece

[2] Department of Genetics, Development and Molecular Biology, School of Biology, Aristotle University of Thessaloniki, Thessaloniki, Greece

[3] Department of Neurology, University Medicine Goettingen and the German Center for Neurodegenerative Diseases (DZNE), Göttingen, Germany

[4] Center for Networked Biomedical Research on Neurodegenerative Diseases (CIBERNED), Instituto de Salud Carlos III, Barcelona, Spain

[5] Bellvitge Biomedical Research Institute (IDIBELL), Hospitalet de Llobregat, Barcelona, Spain

[6] Department of Prosthodontics, School of Dentistry, Aristotle University of Thessaloniki, Thessaloniki, Greece

[7] Department of Biomedical Sciences, International Hellenic University, Thessaloniki, Greece

Prion diseases are fatal transmissible encephalopathies (TSEs), associated with the conversion of the physiological form of the Prion Protein (PrP^{C}) to the disease-associated PrP^{Sc}. Despite extensive research, there has yet to be developed an effective therapeutic and/or preventive intervention. Carnosic acid, a catechol-type diterpene, and its metabolite Carnosol from *Rosmarinus officinalis* have been shown to exhibit anti-oxidant and neuroprotective properties. Since oxidative stress plays a major role in the pathogenesis of Prion disorders, we studied the possible beneficial impact of Carnosic acid and Carnosol in an *in vitro* model of Prion disorders (N2a22L cells) and a *cell-free* Prion amplification assay (RT-QuIC).

In line with their anti-oxidant properties, the expression of genes linked with anti-oxidant response was elevated when N2a22L cells were treated with Carnosic acid or Carnosol. Interestingly, the expression of PRNP, the coding gene for Prion protein, was also upregulated. In addition, both substances demonstrated their effectiveness to neutralize oxidative stress, by decreasing reactive oxygen species (ROS). Carnosic acid or Carnosol treatment of N2a22L cells resulted in a remarkable reduction in the accumulation of the disease-associated form of PrP^{Sc}, as detected by immunoblotting. This effect was validated in *cell-free* assays, demonstrating that Carnosic acid and Carnosol can independently prevent the formation of PrP^{Sc}. Importantly, *cell-free* assays unveiled that these natural products not only prevent the formation of PrP aggregates but can also disrupt already formed aggregates. Our findings suggest that Carnosic acid and Carnosol have pleiotropic effects against Prion diseases, suggesting that they could become important preventative and/or therapeutic agents against Prion and other neurodegenerative diseases.

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Oxidative stress after vaccination for covid 19. A preliminary study

Poimenidou Irini[1], Konstantina Kazeli[1,2], Ioannis Tsamesidis[1,2], Argyrios Gkinoudis[3], Eleni Vagdatli[1], Evgenia Lymperaki[1]*

[1]International Hellenic University, Department of Biomedical Sciences, Thessaloniki, Greece
[2]School of Physics, Faculty of Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece
[3]School of Veterinary Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece

SARS-CoV-2, like other coronaviruses, is an enveloped virus. When S protein binds to the angiotensin-converting enzyme ACE2 which plays a role of membrane receptor, triggers uncontrolled and imbalanced cytokine response and stimulates excessive ROS production. Vaccines are probably the stronger weapon in this war and their effectiveness is significantly influenced by the immunological cellular response to vaccine antigens. After vaccination, innate immune response is temporarily activated. So, its mandatory to understand the vaccine induced immunity and stimulated oxidative stress in order to adjust the number of doses and the time between them to achieve the major benefit. It is known that the first dose of mRNA triggers IgGs antibodies in healthy subject and also after the 2nd dose the antibodies remain for at least 8 months After vaccination, type I interferon induction activates immune response and stimulates excessive ROS production which is associated with inflammation.
At this preliminary study 10 healthy volunteers 5 female and 5 male aged 30-50 years participated. Total ROS were estimated in blood plasma 2 days before and 5 days after completion of the vaccination with both doses for Covid 19. Total ROS were measured by a fluorometric method using the ROS-sensitive probe H_2DCFDA on blood plasma.
The increase of ROS levels, after the first dose of vaccination in all subjects, demonstrates the inflammation which is caused by the antibody's formation after the first dose. This tendency, appears to be controlled and stabilized after the second dose of mRNA vaccines. A fact which is demonstrated by ROS production results in the table below. Finally, we can suggest that ROS can be also measured to confirm antibodies production and could be a helpful biomarker in understanding antibodies production as well as ROS production after vaccination should be a research priority in order to help in the future vaccination strategies.

Vaccine	ROS/a.u before 1st dose	ROS/a.u after 1st dose	ROS/a.u before 2nd dose	ROS/a.u after 2nd dose
MODERNA	22072	28972	28725	23143
MODERNA	15352	20545	20285	16694
MODERNA	20109	8144	14990	17353
MODERNA	14705	19644	19390	20886
MODERNA	21444	23139	28080	17751
PFIZER	14481	16914	17136	11435
PFIZER	17803	24128	25252	17548
PFIZER	21252	2852	30681	20655
PFIZER	18283	19860	20224	17352
PFIZER	16679	17461	18318	16549

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Expression of the glycerophospholipid and triacyglyceride synthesis enzyme AGPAT4 is upregulated in cancer cells under hypoxic conditions.

Angeliki Karagiota[1,2], Konstantina Paraskeva[2], Christina Arseni[1], Ilias Mylonis[1], George Simos[1], Georgia Chachami[1], and Efrosyni Paraskeva[2]

[1]*Laboratory of Biochemistry, Faculty of Medicine, University of Thessaly, Larissa, Greece*

[2]*Laboratory of Physiology, Faculty of Medicine, University of Thessaly, Larissa, Greece*

Hypoxia inducible factor-1 (HIF-1) supports survival of normal cells under low oxygen concentration and cancer cells in the hypoxic tumor microenvironment. To this end, HIF-1 reprograms lipid metabolism, contributing significantly to increased proliferation and chemoresistance of cancer cells. Among its target genes involved in lipid metabolism, HIF-1 mediates the induction of acylglycerolphosphate acyltransferase-2 (AGPAT2), an enzyme that catalyzes the formation of phosphatidic acid (PA), the second step of the triacylglyceride (TAG) biosynthesis pathway. AGPAT2 belongs to a family of five isoforms AGPAT1-5, that all catalyze the same biochemical reaction, but have each unique physiological and pathological roles, dependent on their distinct tissue distribution, substrate specificity and expression regulation. Recently, AGPAT isoform expression has been associated with cancer progression, suggesting that they may be used as biomarkers and therapeutic targets for cancer diagnosis and treatment. Using hepatocarcinoma (Huh7), lung adenocarcinoma (A549 and H1299) and breast cancer (MCF7) cell lines, we now show, that, in addition to AGPAT2, the expression of AGPAT4 mRNA and protein is also upregulated under hypoxic conditions. Induction of AGPAT4 expression is HIF-1-dependent, as it is prevented upon *HIF1A* knockout in HeLa cells. Furthermore, bioinformatics analysis of publicly available cancer patient data, shows that the expression of AGPAT4 is positively correlated with the expression of both *HIF1A* and a hypoxia gene signature, in human breast invasive carcinoma (BRCA), cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), liver hepatocellular carcinoma (LICH) and lung adenocarcinoma (LUAD) tumors, corresponding to the cell lines analyzed above. Importantly, in two of these tumor types, CESC and LICH, high AGPAT4 expression is associated with negative prognostic outcome, highlighting the importance of hypoxia-induced upregulation of AGPAT4 for human patient tumor growth.

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The synergy between EGFR and E2/ERβ signaling governs morphological characteristics, functional properties, and aggressiveness in triple-negative breast cancer

Konstantina Kyriakopoulou[1], Elena Kefali[1], Zoi Piperigkou[1], Christoph Riethmüller[2], Burkhard Greve[3], Marco Franchi[4], Martin Götte[5], Nikos K. Karamanos[1]

[1]Biochemistry, Biochemical Analysis & Matrix Pathobiology Res. Group, Lab. of Biochemistry, Department of Chemistry, University of Patras, 26504, Greece

[2]Serendip GmbH, Center for Nanotechnology, Heisenbergstr. 11, D48149, Münster, Germany, Münster, Germany

[3]Department of Radiotherapy–Radiooncology, University Hospital Münster, Albert-Schweitzer-Campus 1, A1, 48149, Münster, Germany

[4]Department for Life Quality Study, University of Bologna, 47921 Rimini, Italy

[5]Department of Gynecology and Obstetrics, University Hospital Münster, Albert-Schweitzer-Campus 1, D11, 48149, Münster, Germany

Triple negative breast cancer (TNBC) is characterized by increased metastatic potential and lower overall survival rates. Metastasis is highly complex and includes extracellular matrix (ECM) remodeling, epithelial-to-mesenchymal transition (EMT) activation and cytoskeleton reorganization. EMT has been closely linked with the emergence of cancer stem cells (CSCs) and subsequent acquisition of chemoresistance. In TNBC, epidermal growth factor receptor (EGFR)-dependent signaling induces the expression of distinct EMT-related molecules. We have recently reported that the EGFR/estrogen receptor β (ERβ) crosstalk is crucial for the regulation of cell-matrix interactions and invasion of TNBC cells. Here, the regulatory role of the EGFR – E2/ERβ axis in the morphology, functional properties and aggressive characteristics of TNBC cells was evaluated. ERβ-suppressed and control MDA-MB-231 cells subjected to downstream EGFR inhibition and/or estradiol stimulation were studied by SEM, AFM and immunofluorescence microscopy as to assess alterations in cell morphology. Cell viability, cell cycle progression, cell migration, *in vitro* angiogenesis, colony and spheroid formation were evaluated as functional parameters, whereas EMT and cancer stem cell markers were studied by qPCR, immunofluorescence microscopy and flow cytometry. EGFR inhibition resulted in an overall suppression of these functional markers of TNBC aggressiveness, which occurred in an ERβ-dependent manner. At the molecular level, these changes could be attributed to a reduction of markers of EMT and stemness, most notably reduced expression of Notch signaling constituents and syndecan-1. Our study highlights the importance of EGFR signaling as a key effector of aggressiveness and stemness in an ERβ-dependent way in TNBC.

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Structural and functional characterization of recombinant polypeptides of Hepatitis E Open Reading Frame 1 (ORF1)

Maria D. Politi[1], Angelo Galo[1], Georgios Bouras[1], Maria Birkou[1],Dioni Ntonti[1], Bruno Coutard[2], Georgios A. Spyroulias[1*]
[1]*Department of Pharmacy, University of Patras, Patra, Greece*
[2]*AFMB, UMR 7257 CNRS/ University of Merseille, Merseille CEDEX 9, France*
*email: g.a.spyroulias@upatras.gr

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 us 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' UTR.
Our study focuses on ORF1, which encodes the non-structural proteins, important for the virulence. ORF1 has several conserved putative functional domains including a macro domain (MD), a methyltransferase (MeT) and a papain-like cysteine protease (PCP). In general, the MDs have conserved structural folds of about 160-180 amino acids, which can bind ADP-ribose, and its derivatives. HEV MD is responsible for many cellular processes during viral infection, such us de-MARylation and/or de-PARylation. On the other hand, HEV MeT catalyzes the transfer of methyl group from S-adenosyl methionine to GTP, to yield m7GTP. In addition, PCP is important for HEV polyprotein processing and has been associated with deISGylation activity which may be essential for invading cellular antiviral pathways2. In the present study, the expression and the purification of the three proteins from ORF1 are described. Specifically, a wide variety of experimental conditions were tested for different length recombinant polypeptides of ORF1 products, to identify polypeptides that renders stability to the protein domains and are suitable for conformational, structural NMR spectroscopy studies and biological procedures.

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WISP-1 as a potential regulator of GBM aggressiveness

Zoi Karagiorgou, Panagiotis Fountas, Giannis Giotis, Dimitra Manou, Alexios J. Aletras, Achilleas D. Theocharis
Biochemistry, Biochemical Analysis & Matrix Pathobiology Research Group, Laboratory of Biochemistry, Department of Chemistry, University of Patras, Greece

Glioblastoma (GBM) is the most common aggressive primary brain tumor with the median overall survival to be 15 months. The tumor microenvironment including stromal cells and extracellular matrix (ECM) is undergoing extensive alterations in many tumors, as well as in GBM, leading to tumor growth. Serglycin is a proteoglycan (PG) that emerged to play a key role in glioblastoma aggressiveness. Even though serglycin was discovered in intracellular compartments, its secreted form participates in maintaining the proteolytic and inflammatory potential of tumor cells via regulation of the biosynthesis, secretion and activity of various components of the ECM. WISP-1 is a secreted cysteine-rich growth factor and signals through integrins. Despite its vital role in embryonic stem cells proliferation, differentiation, apoptosis and adhesion, WISP-1 has been directly linked to the progression of various tumors. WISP-1 expression is found upregulated in GBM, compared to non-malignant tissues and cells, while its suppression resulted in reduced cell proliferation, migration, invasion and stemness phenotype. WISP-1 can regulate the expression of various downstream targets involving in tumor progression including MMP-9, MMP-2, VEGF-A, IL-12 and β-catenin. Our laboratory has generated LN-18 GBM cells with suppressed levels of serglycin (LN-18^{shSRGN}) characterized by reduced proliferation and migration rates, as well as tumorigenesis *in vivo*. LN-18^{shSRGN} cells exhibit reduced proteolytic and inflammatory dynamic, including lower mRNA levels of WISP-1. Our study is focused on the investigation of the role of serglycin to regulate GBM cell aggressiveness via modulation of WISP-1 levels and activity.

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Microbiome in chronic kidney disease (CKD): an omics perspective

Sonnal Lohia*, Jerome Zoidakis, Antonia Vlahou
Proteomics Laboratory, Biomedical Research Foundation Academy of Athens, Greece
* slohia@bioacademy.gr

Gut microbiota plays an essential role in maintaining host energy homeostasis and gut epithelial integrity. CKD is predominant in 10% of world's adult population, as a silent epidemic. Alterations in gut microbiota and specifically production of metabolites causing uremic toxicity by intestinal bacteria are associated with CKD onset and progression. We present the available omics studies that explore the connection between CKD and gut microbiome. A predominance of metabolomics studies (n= 12) over transcriptomics (n=1) and proteomics (n=6) was observed. Collectively, findings include an observed enrichment of *Eggerthella lenta, Enterobacteriaceae* and *Clostridium* spp., and a depletion in *Bacteroides eggerthii, Roseburia faecis* and *Prevotella* spp. occuring in CKD models. Uremic toxins produced in the gut cause oxidative stress, inflammation and fibrosis in the kidney leading to CKD. Bacteria related to CKD are also involved in butyrate production and mucin degradation. Strong links between CKD and gut microbial dysbiosis suggest potential therapeutic strategies to prevent CKD progression.

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DHEA and its synthetic derivative BNN27 affect the pro-inflammatory phenotype of human adipocytes and mouse 3T3L1 adipocytes

Michalis Deiktakis[1], Dimitris Frantzetis[1], Elias Athanasakis[2], John Charalampopoulos[3,4], Christos Tsatsanis[1,4], Achille Gravanis[3,4], Andrew N. Margioris[1] and Eirini Dermitzaki[1*]

[1]*Department of Clinical Chemistry, School of Medicine, University of Crete, Greece*
[2]*Department of Surgery, University General Hospital of Heraklion, Heraklion, Crete, Greece*
[3]*Department of Pharmacology, School of Medicine, University of Crete, Greece*
[4]*Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology Hellas, Heraklion, Crete, Greece*

Introduction
The hallmark of obesity is the hypertrophic and hyperplastic adipose tissue, especially in the abdominal area. Therein, white adipocytes along with infiltrating macrophages give rise to chronic low-grade systemic inflammation which results in metabolic dysfunction and all in all deterioration of the organism's metabolic health.
Dehydroepiandrosterone (**DHEA**) is a steroid hormone synthesized mainly by adrenals and, surprisingly, large concentrations of this circulating hormone reside in white adipose tissue (WAT) modulating its physiology. However, DHEA levels are reduced in ageing subjects and the supplementation with DHEA is limited due to its estrogenic action. Thus, the possible use of synthetic analogs of DHEA, like (20R)-3β,21-dihydroxy-17α,20-epoxy-5-pregnene (**BNN27**), may serves as promising pharmacological candidates for the study of obesity.
The purpose of this study is to elucidate the role of DHEA and BNN27 in the inflammatory profile of adipocytes.
Materials and methods
Visceral white adipose tissues were obtained from patients undergoing general surgery from PAGNI hospital. WAT was processed to obtain distinct populations of mature adipocytes. Moreover, the mouse pre-adipocytes 3T3L1 were differentiated *in vitro* to white adipocytes. DHEA and BNN27 were added at different time points for all experimental groups, where lipid accumulation was measured by Oil-Red-O staining, levels of inflammatory markers by ELISA and mRNA levels of adiponectin by qPCR.
Results
DHEA and BNN27 decreased intracellular lipid accumulation in human mature adipocytes and in 3T3L1 cells during the adipogenesis process. Moreover, both molecules induced a significant reduction in the secretion of proinflammatory chemokines and cytokines. Finally, DHEA and BNN27 increased the mRNA levels of adiponectin.
Conclusion
The role of DHEA in the inflammatory profile of adipocytes is indeed beneficial. Interestingly, the BNN27 exerts similar positive action in both human and murine adipocytes, rendering it a potential new pharmacological agent in the battle of obesity.

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Overlapping roles of yeast transporters Aqr1, Qdr2, and Qdr3 in amino acid excretion and cross-feeding of lactic acid bacteria

George C. Kapetanakis[a], Christos Gournas[b], Martine Prévost[c], Isabelle Georisd,[e], Bruno Andréa,[e]
[a] *Molecular Physiology of the Cell, Université Libre de Bruxelles (ULB), Biopark, Gosselies, Belgium*
[b] *Microbial Molecular Genetics Laboratory, Institute of Biosciences and Applications, National Centre for Scientific Research "Demokritos", Agia Paraskevi, Greece*
[c] *Structure et Fonction des Membranes Biologiques, Université Libre de Bruxelles (ULB), Brussels, Belgium*
[d] *Transport of amino acids, sensing and signaling in eukaryotes, LABIRIS, Brussels, Belgium*
[e] *These authors equally contributed to this work*

Microbial species occupying the same ecological niche or codeveloping during a fermentation process can exchange metabolites and mutualistically influence each other's metabolic states. For instance, yeast can excrete amino acids, thereby cross-feeding lactic acid bacteria unable to grow without an external amino acid supply. The yeast membrane transporters involved in amino acid excretion remain poorly known.
Using a yeast mutant overproducing and excreting threonine (Thr) and its precursor homoserine (Hom), we show that the Aqr1, Qdr2, and Qdr3 proteins of the Drug H+-Antiporter Family (DHA1) family mediate excretion of both amino acids. We further investigated Aqr1 as a representative of these closely related amino acid exporters. In particular, structural modeling and molecular docking coupled to mutagenesis experiments and excretion assays enabled us to identify residues in the Aqr1 substrate-binding pocket that are crucial for Thr and/or Hom export. We then co-cultivated yeast and Lactobacillus fermentum in an amino-acid-free medium and found a yeast mutant lacking three DHA1 family members to display a reduced ability to sustain the growth of this lactic acid bacterium, a phenotype not observed with strains lacking only one of these transporters. This study highlights the importance of DHA1 transporters in amino acid excretion and reveals the role of these proteins in mutualistic interaction with lactic acid bacteria.

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SuptoxR and SuptoxR2.0: Specialized *Escherichia coli* strains for recombinant membrane protein production at high yields

Eleni Vasilopoulou[1,2], Dimitra Gialama[1], Myrsini Michou[1,2], Charalampos Kapsalis[1], Aristeidis Michoglou-Sergiou[1], Fragiskos N. Kolisis[3], Georgios Skretas[1]*
[1]*Institute of Chemical Biology, National Hellenic Research Foundation, Athens, 11635 Greece*
[2]*Department of Biochemistry and Biotechnology, University of Thessaly, Larissa 41500, Viopolis, Greece*
[3]*Laboratory of Biotechnology, School of Chemical Engineering, National Technical University of Athens, Athens 15780, Greece*
**e-mail: gskretas@eie.gr*

Membrane proteins (MPs) are basic components of cell membranes where they perform highly important functions. Their crucial role is highlighted by the fact that in both prokaryotes and eukaryotes they are encoded by 20-30% of all genes and due to their key location and their multiple functions, they constitute more than half of all known targets for drug development. As a result, there is a huge need for access to large amounts of MPs in order to expedite the discovery of new pharmaceuticals that target such proteins, through the detailed characterization of their structure and function. The required quantities of MPs are typically produced recombinantly in heterologous hosts such as *Escherichia coli* (*E. coli*), mainly because of their low natural abundance. In case of heterologous expression though, recombinant MP production in bacteria is accompanied with severe cytotoxicity for the host, making their use particularly difficult. Towards this direction, in previous work we managed to face this need and we engineered a genetically modified *E. coi* strain, SuptoxR, which overexpresses the gene, encoding for RraA, an inhibitor of the mRNA-degrading activity of *E.coli* RNase E. This strain has been proven particularly effective in suppressing the toxicity, which is often generated during the process of MP overexpression, while at the same time, it markedly increased the cellular accumulation of recombinant MPs of prokaryotic and eukaryotic origin. Herein, we evaluate a set of homologous RraA proteins from bacteria and plants for their ability to suppress MP-induced toxicity and enhance the productivity of recombinant MPs. This process led to the identification of several homologous suppressors, and enhancers of MP production. Intriguingly, some of them were found capable of enhancing bacterial MP production more effectively than the *E.coli* RraA of SuptoxR. Based on these results, we have developed second-generation SuptoxR strains, termed SuptoxR2.0, which can achieve even further enhanced levels of MP production in *E.coli*.

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Canine Degenerative Myelopathy: A pilot comparative serum protein analysis of diseased and healthy animals

Stefania-Iliana Tziola[1,2,3], Konstantina Psatha[1,3,4], George Stamatakis[5], Rania D. Baka[6], Martina Samiotaki[5], Alexandros Triantafyllidis[2], Zoe Polizopoulou[6]*, Michalis Aivaliotis[1,3,4]*

[1]Laboratory of Biological Chemistry, School of Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece

[2]Laboratory of Genetics, Development and Molecular Biology, Department of Biology, Aristotle University of Thessaloniki, Thessaloniki, Greece

[3]Functional Proteomics and Systems Biology (FunPAth), Center for Interdisciplinary Research and Innovation(CIRI-AUTH),Thessaloniki, Greece

[4]Institute of Molecular Biology and Biotechnology, FORTH, Heraklion, Greece

[5]Institute for Bioinnovation, Biomedical Sciences Research Center "Alexander Fleming", Vari, Attica, Greece

[6]Diagnostic Laboratory, School of Veterinary Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece

*Correspondence: aivaliotis@auth.gr, poliz@vet.auth.gr

Introduction: Canine degenerative myelopathy (DM) is a progressive, neurodegenerative disorder of the spinal cord that mainly affects dogs older than eight years[1].Until now, DM represents the first naturally occurring animal model of human Amyotrophic Lateral Sclerosis (ALS)[2]. Proteomics has already been established as a valuable tool for the detection of specific biomarkers in patients' body fluids[3].Interestingly, ALS has not yet received much attention in terms of mass spectrometry (MS) -based proteomics analysis[2].

Materials and Methods: Six canine serum samples (3 from healthy and 3 from DM-affected dogs) were prepared and analyzed using in solution tryptic digestion, followed by nLC-ESI-MS/MS analysis. Subsequently, bioinformatic analysis was conducted using MaxQuant and Proteome Discoverer tools. Gene Ontology (GO; molecular functions, biological processes, cellular compartment) and pathway analysis of the identified proteins was retrieved from UniProt and String.

Results: 226 proteins were identified in the control and DM samples. 12 of them were identified only in the DM-affected dogs. Functional analysis of these 12 proteins, demonstrated that the more significantly expressed and related to the disease proteins were associated with inflammation and neurodegeneration (SAA1, lysozyme, LBP[4,5]). Proteins identified with different abundance, having a pivotal role in disease's onset and progression were GPX3, ApoE, ApoD, and the complement cascades' components C1qB and C1qC[6,7,8].

Discussion-Conclusions: Canine DM is associated with alterations in the expression levels of the identified proteins. To the authors' knowledge, this is the first time proteomic analysis was performed in serum samples of DM-affected dogs. Further research is needed in order to validate the results of the present study as diagnostic or prognostic biomarkers of canine DM and human ALS.

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Non-steroidal Selective Glucocorticoid Receptor Agonists: the example of AZD9567

Dimitra Siakouli[1], Georgios Panagiotou[1], Vassiliki Ganou[1], Eleni-Fani Gkotsi[1], Aristotelis Chatziioannou[2], Olga Papadodima[1], Michael N. Alexis[1], Dimitra J. Mitsiou[1]*
[1]Institute of Chemical Biology, National Hellenic Research Foundation, Athens, Greece
[2]Center of Systems Biology, Biomedical Research Foundation of the Academy of Athens, Greece
**e-mail: dmitsiou@eie.gr*

Glucocorticoids (GCs) are essential steroid hormones widely used as potent anti-inflammatory drugs; however, their chronic clinical use is often accompanied by adverse side effects. The anti-inflammatory action of GCs is exerted through the glucocorticoid receptor (GR) in part by antagonizing the pro-inflammatory nuclear factor kappa B (NF-kB) whereas the majority of side effects are assumed to be mediated by transactivation of GR target genes. Identification of selective GR agonists (SEGRAs) that preferentially favor transrepression of pro-inflammatory NF-kB target genes over transactivation of genes associated with undesirable effects is expected to improve the therapeutic potential of GCs.
We have previously identified two 1,3-benzothiazole analogs as novel non-steroidal SEGRA [1]. In the present study, we analyzed the function of AZD9567, a potent SEGRA with improved side effect profile [2]. We used different cell-based assays and showed that AZD9567 displays full efficacy in the transrepression of key pro-inflammatory genes and partial efficacy in the transactivation of GR targets genes, as compared to dexamethasone (Dex, a classical GR agonist). Further, we used RNA-sequencing to identify the global transcriptome profile of AZD9567, in comparison to that of Dex, in RAW 264.7 mouse macrophages. Our data revealed that AZD9567 induces a transcriptomic profile distinct from that of Dex in RAW 264.7 cells. Comparison of the transcriptomic profiles of AZD9567 and Dex in RAW 264.7 cells treated with lipopolysaccharide (LPS) to elicit an inflammatory response revealed three types of anti-inflammatory gene signatures: 'AZD9567- and Dex-specific', 'only AZD9567-specific' and 'only Dex-specific'. Bioinformatic analysis of the 'AZD9567- and Dex-specific' signature indicated that the LPS-induced genes that are repressed by both Dex and AZD9568 include key pro-inflammatory genes that play critical role in the anti-inflammatory function of GR. Our data reinforce the importance of the development of selective GR agonists able to confer improved anti-inflammatory effects.

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Hypoxia induces HIF-independent alteration of nuclear envelope architecture by ROS-mediated Lamin B Receptor redistribution

Chrysa Taze, Eleni Georgatsou, George Simos, Ilias Mylonis*
Laboratory of Biochemistry, Faculty of Medicine, University of Thessaly, 41500 Biopolis, Larissa, Greece
**e-mail: mylonis@med.uth.gr*

Cellular adaptation to oxygen deprivation (hypoxia) primarily involves transcriptional reprogramming mediated by Hypoxia Inducible Factors. However, cells also respond to hypoxia by lesser-known transcription-independent processes that occur early after hypoxia. These include epigenetic marking of chromatin, alterations in mRNA processing and cellular architecture. Nuclear envelope comprises the outer and inner nuclear membrane (INM), which is underlain by a filamentous meshwork, the nuclear lamina. Lamin B Receptor (LBR) is an integral INM protein composed of hydrophilic N-terminal and C-terminal tails that protrude into the nucleoplasm and eight membrane-spanning segments. The N-terminal domain is targeted by SRPK1 kinase and serves as a phosphorylation-controlled interaction platform for proteins such as lamin B and heterochromatin components. We report that in cancer cells (MCF7, HeLa) the shape and smoothness of nuclear envelope is altered shortly after onset of hypoxia, suggesting HIF-independent changes in nuclear plasticity. At the same time, biochemical fractionation and immunofluorescence microscopy experiments show that hypoxia reversibly redistributes LBR alongside the nuclear periphery. Parallelly, hypoxia increased the presence of cytoplasmic chromatin fragments (CCF), which were marked by the presence of LBR. To explain these phenomena, we performed immunoprecipitation experiments the results of which show that hypoxia reduces both LBR phosphorylation and interaction with Lamin B and SRPK1 kinase. Furthermore, SRPK1 is shown to translocate from the nucleoplasm and nuclear matrix into the cytoplasm, explaining the reduction of LBR phosphorylation. This rearrangement of nuclear envelope components under hypoxia was reversed by treatment with the antioxidant n-acetylcysteine and phenocopied by rotenone, suggesting that it is driven by the increased mitochondrial production of reactive oxygen species (ROS) at the early stages of hypoxia. Overall, our data suggest that the cellular response to hypoxia entails ROS-driven and LBR-mediated changes in the architecture of the nuclear periphery which may be important for reprogramming chromatin status and gene expression.

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Discovery of novel industrially relevant enzymes through high-throughput metagenomic analysis and functional screening

Dimitra Zarafeta[1], Aristotelis Chatziioannou[1,5], Evangelia D. Chrysina[1], Sergey Gavrilov[2], Theodoros Koutsandreas[1,5], Ilya Kublanov[2], Efthymios Ladoukakis[3], Danai Moschidi[1], Xu Peng[4], Eleftherios Pilalis[1,5], Zalan Szabo[6], N. Kolisis[3*], Georgios Skretas[1*]

[1]Institute of Chemical Biology, National Hellenic Research Foundation, Athens, Greece
[2]Winogradsky Institute of Microbiology, Russian Academy of Sciences, Moscow, Russia
[3]School of Chemical Engineering, National Technical University of Athens, Greece
[4]Danish Archaea Centre, Copenhagen University, Denmark
[5]e-NIOS Applications PC, Athens, Greece
[6]MicroDish B.V., Utrecht, Netherlands

Hydrolytic enzymes are biocatalysts widely used in biotechnology. Production of fine chemicals and pharmaceuticals, functional food additives and biofuel production through biomass degradation are some of the applications that rely on enzymatic catalysis performed by hydrolases. Despite their broad range of applicability, a limited number of these enzymes are currently being used in industrial processes. This is due to the fact that conventional biocatalysts perform poorly in industrial setups that involve high temperatures and harsh reaction conditions. Low enzyme performance impacts negatively the feasibility of the application meant to serve and the cost and availability of the end product. This has created an increasing market demand for enzymes with specific industrially relevant characteristics, such as stability at elevated temperatures and in the presence of denaturing factors, which will allow their incorporation into cost-efficient biotechnological processes. Extremophilic organisms are a rich potential source of such enzymes which remains largely unexplored due to the fact that their vast majority (>99%) cannot be cultured using standard laboratory techniques. In this work, we present how we bypass this bottleneck and gain access to the huge protein space of extremophiles in order to discover novel hydrolases suitable for industrial application. Through the development of an automated bioinformatics analysis platform and functional high- throughput screening approaches we have been able to screen metagenomic next generation sequencing data and metagenomic DNA material originating from extreme environment located all around the world. High-throughput screening of billions of ORFs have led us to the identification of genes that encode for enzymes with desirable enzymatic activities. Recombinant protein synthesis, functional assays and structural analysis have led us to the identification and characterization of novel enzymes such as thermostable esterases, that we showcase herein, with interesting biochemical profiles and traits that render them candidate biocatalysts for various biotechnological applications.

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Heterologous expression of the C-terminal domain (CBM20) of the human Starch binding domain-containing protein 1 (STBD1)

Athanasios Toulios, Eirini Theodosiou, Serafeim Alexopoulos, George Stravodimos, Christina Drakou, Vasiliki Skamnaki*
Laboratory of Structural and functional Biochemistry, Department of Biochemistry and Biotechnology, University of Thessaly, Larissa, Greece
** Corresponding author: vskamnaki@bio.uth.gr*

Starch binding domain-containing protein 1 (STBD1) is a carbohydrate-binding protein that is expressed predominantly in muscle and liver, and it is implicated in the metabolism and cellular trafficking of glycogen.[1,2] Due to the interaction with GABARAPL1 through the Atg8-family interacting motif (AIM), it was proposed as a selective autophagy receptor for glycogen (glycophagy), mediating the lysosomal glycogen transport in the liver.[3,4] Moreover, recent studies indicated that STBD1 induces the reorganization of the ER, affects the ER-mitochondria association and promotes the glycogen clustering to organized smooth ER (OSER) structures, in response to ER stress activation.[5,6]
The human STBD1 has 358 amino acids and a MW of 39 KDa. The structural information on STDB1 remains elusive to date. Towards this direction, by using bioinformatics tools we identified the C-terminal carbohydrate binding CBM20 domain (aa 258-358) of STBD1. The vector pGEX-6P-1_CBM20 was constructed for its heterologous expression as GST fused protein in *E.coli*. Several expression tests were performed in order to optimize the conditions for the production of soluble protein in high yields, suitable for further biochemical and structural studies.

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Identification of protein complexes to improve lignocellulosic biomass

Varvara Podia[1], Ioannis Isaioglou[2], George Kapolas[1], Mike Karampelias[2], Dimitra Milioni[3], Polydefkis Hatzopoulos[3], Athanassios Velentzas[4], Dimitrios Stravopodis[4], Kosmas Haralampidis[1]*

[1] National and Kapodistrian University of Athens, Department of Biology, Section of Botany, Greece

[2]King Abdullah University of Science and Technology (KAUST), Division of Biological and Environmental Sciences and Engineering, Thuwal, Saudi Arabia

[3] Agricultural University of Athens, Department of Biotechnology, Greece

[4]National and Kapodistrian University of Athens, Department of Biology, Section of Cell Biology & Biophysics, Greece

Humanity faces an unprecedented challenge in terms of energy demand. Fossil fuel reserves are being depleted, while combustion of these energy sources increases the greenhouse effect, leading to unpredictable climate change. In the years to come, CO_2 emissions must be significantly reduced worldwide, in order to overcome these concerns. Thus, the transition to more environmentally friendly renewable energy sources is imperative. Secondary walls are the most abundant biomass produced by vascular plants and are important raw materials for many industrial uses. Understanding the mechanisms of cell wall biogenesis and plant secondary growth is of significance and has far-reaching implications in genetic engineering of plant biomass, better suited for various end uses, such as biofuel production. Vascular tissue differentiation and secondary growth involves mechanisms such as proliferation of cambium, differentiation and modification of the cellular content and thickening of cell walls. Herein, we intended to identify the putative interaction partners and complexes of VPNB1 and APRF1 by BiFC and Co-IP. To achieve this goal, we created various transgenic lines containing the coding regions of *VPNB1* and *APRF1* in translational fusion with flag peptide, YFP and/or GFP proteins. We also used VISUAL to produce vascular system cell cultures. Our data revealed that both VPNB1 and APRF1 show a dual localization to both the nucleus and the cytoplasm. The VISUAL vascular cell system resulted in an extremely broad induction of *VPNB1* expression, confirming the crucial role of the protein in vascular cell differentiation. Furthermore, we identified by Co-IP and FLAME software analysis several APRF1 interacting proteins, including HSPs. BiFC experiments in *Nicotiana benthamiana* leaves confirmed the interaction of APRF1 with the HSP90.1 and HSP90.2 proteins.

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P90

Investigating the rebound effect of anti-RANKL treatment discontinuation in the TgRANKL osteoporosis mouse model

Vagelis Rinotas[1,2], Dimitra Ntouskou[1], Melina Dragolia[2], Vassilis Ntafis[2], Maria Yavropoulou[3], Athanasios Anastasilakis[4], Polyzois Makras[5], Eleni Douni[1,2*]

[1] Laboratory of Genetics, Department of Biotechnology, Agricultural University of Athens, Athens, Greece,
[2] Biomedical Sciences Research Center "Alexander Fleming", Vari, Greece,
[3] Endocrinology Unit, 1st Department of Propaedeutic and Internal Medicine, Medical School, National and Kapodistrian University of Athens, Athens, Greece.
[4] Department of Endocrinology, 424 General Military Hospital, Thessaloniki, Greece.
[5] Department of Endocrinology and Diabetes and Department of Medical Research, 251 Hellenic Air Force & VA General Hospital, Athens, Greece.
**Corresponding Author*

Denosumab (Dmab), a monoclonal antibody against RANKL, which is a key regulator of osteoclastogenesis, is effective as an antiresorptive agent in the treatment of post-menopausal osteoporosis. Notably, Dmab discontinuation is associated with a severe rebound effect that leads to a rapid bone loss and increased fracture risk within one year after last dose. However, Dmab is ineffective in osteoporotic mouse models since it cannot bind to mouse RANKL. In the current study, we modelled Dmab discontinuation in our TgRANKL osteoporotic transgenic mice that overexpresses human RANKL. The experimental groups included: i) wild-type (WT) mice untreated, ii) TgRANKL untreated, iii) TgRANKL treated with Dmab for 18 weeks, and iv) TgRANKL treated with Dmab for 6 weeks then discontinuation for 12 weeks (discontinuation group). Our results showed that TgRANKL femurs displayed significant trabecular bone loss compared to WT, while continuous Dmab treatment of TgRANKL mice completely inhibited bone resorption and increased trabecular bone volume above the values of WT mice. Notably, in the discontinuation group we detected a rapid bone loss as estimated by the significantly reduced trabecular bone volume, which reached similar levels with the untreated osteoporotic TgRANKL mice. Gene expression analysis for osteoclastogenic (*Dcstamp*, *Ctsk*) and osteoblastogenic markers (*Runx2*, *Alp*) confirmed the various treatment effects on the skeletal system. Furthermore, we examined the effects of Dmab discontinuation in bone marrow adiposity (BMA) since osteoporosis is related with increased BMA. Histological examination of distal femurs revealed a dramatic increase of BMA in TgRANKL untreated mice that was fully reversed upon Dmab treatment. Interestingly, BMA reappeared in the Dmab discontinuation group but to a lesser degree compared to TgRANKL untreated mice. In conclusion, we established a Dmab discontinuation mouse model to investigate the underlying molecular mechanisms and evaluate various therapeutic approaches following Dmab discontinuation at the preclinical level.

P91

An enriched polyphenolic extract obtained from the by-product of *Rosa damascena* hydrodistillation activates antioxidant and proteostatic modules

Evanthia Dina[1], Aimilia D. Sklirou[2], Sofia Chatzigeorgiou[2], Maria S. Manola[2], Antigoni Cheilari[1], Xanthippi P. Louka[2], Aikaterini Argyropoulou[1], Nikos Xynos[1], Alexios-Leandros Skaltsounis[1], Nektarios Aligiannis[1], Ioannis P. Trougakos[2]

[1] *Department of Pharmacognosy and Natural Products Chemistry, Faculty of Pharmacy, National and Kapodistrian University of Athens, Panepistimiopolis Zografou, Athens, Greece.*

[2] *Department of Cell Biology and Biophysics, Faculty of Biology, National and Kapodistrian University of Athens, Panepistimiopolis, Athens, Greece*

Background: Prolonged maintenance of proteome stability and functionality (proteostasis) is of emerging significance in aging retardation and healthspan.
Purpose: An enriched polyphenolic extract obtained from the hydrodistillation of rose petals was tested for its capacity to activate the proteostasis network modules, and thus modulate health- and/or lifespan at the cellular and whole organism level.
Methods: The aqueous extract that remained after the hydrodistillation of *Rosa damascena* petals, was processed with a polystyrene-FPX66 adsorption resin and sequentially fractionated by FCPC. NMR and UHPLC-HRMS analyses revealed the presence of 28 metabolites, mainly glycosides of kaempferol and quercetin.
Results: The extract showed high *in vitro* antioxidant activity and was not toxic in normal human skin fibroblasts, while it promoted the upregulation of NRF2-induced antioxidant genes and main proteostatic modules. Consistently, supplementation of this extract in *Drosophila* flies' culture medium induced a cncC/NRF2-mediated upregulation of antioxidant and proteostatic modules. Prolonged administration of the extract in flies' culture medium was not toxic and did not affect food intake rate or fecundity; also, it delayed the age-related decline of stress tolerance and locomotion performance (neuromuscular functionality) and dose-dependently extended flies' lifespan.
Conclusion: Our findings indicate that the enriched polyphenolic extract obtained from the residue of *R. damascena* hydrodistillation activates cytoprotective cellular modules that, likely, contribute to its potential anti-aging properties.

P92

Investigation of the role of tumor suppressor CYLD in mammary epithelial cell response to DNA damage by chemotherapeutic topoisomerase II inhibitors

Athanasios Siasiaridis*, George Mosialos
Aristotle University of Thessaloniki, Department of Genetics, Development and Molecular Biology, School of Biology, Greece

CYLD is a deubiquitinating enzyme that cleaves preferentially K63- and M1-linked polyubiquitin chains. CYLD is downregulated in various types of cancer including breast cancer. We have shown that CYLD suppresses epithelial to mesenchymal transition (EMT) in human mammary epithelial cells at least in part by suppressing the TGFbeta pathway. Since EMT predisposes cells to DNA damage because of activation of the TGFbeta pathway we tested the hypothesis that CYLD-deficient mammary epithelial cells are more susceptible to DNA damage. To examine our hypothesis, we treated wild type and CYLD-deficient MCF10A cells with etoposide and doxorubicin, two topoisomerase II inhibitors used as chemotherapeutic agents. The etoposide and doxorubicin treatment results in DNA double strand breaks which accumulate and lead to errors in DNA synthesis promoting apoptosis. The DNA damage was assessed via quantification of γH2AX, the phosphorylated form of histone H2AX on Ser139. H2AX becomes phosphorylated as a reaction to DNA double strand breaks, and it is a common marker to assess the extent of DNA damage in cells. The levels of γH2AX were measured via immunoblotting and the ratio γH2AX/H2AX was calculated for each sample. Etoposide was also used to assess the kinetics of recovery from DNA damage in CYLD-deficient and control cells. The induction of DNA damage was successful with both agents. Wild type and CYLD deficient cells showed an increase in the amount of γH2AX and the treated samples showed an elevated γH2AX/H2AX ratio compared to DMSO-treated samples. Our results showed that the ablation of CYLD did not affect the accumulation of DNA damage or the kinetics of recovery from DNA damage. We are currently investigating the potential involvement of CYLD in other types of DNA damage and relevant responses.

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LPA as a proinflammatory stimulus on renal tubular epithelial cells

Georgia Antonopoulou[1], Christiana Magkrioti[1], Vicky Pliaka[2], Theodore Sakellaropoulos[2], Dionysios Fanidis[1], Leonidas G Alexopoulos[2,3], Vassilis Aidinis[1]

[1]Institute for Bioinnovation, Biomedical Sciences Research Center Alexander Fleming, Athens, Greece
[2]ProtATonce Ltd., Athens, Greece
[3]School of Mechanical Engineering, National Technical University of Athens, Zografou, Greece

Renal tubular epithelial cells (TECs) are lining the nephrons, the functional units of the kidneys. They perform the selective transport of minerals, organic compounds and water into and out of the tubular fluid. TECs are vulnerable to injuries and, therefore, amenable to apoptosis, mitochondrial dysfunction, oxidative stress, senescence and proinflammatory cytokine secretion resulting in abrogation of the tubular function [1]. Upon multiple injuries repair mechanisms become maladaptive and lead to tubulointerstitial fibrosis. Based on TECs crucial role on renal fibrosis we wanted to discover new stimuli that could evoke responses on these cells, among them lysophosphatidic acid, LPA. LPA is a bioactive lipid mediator that triggers several physiological events such as cell proliferation, survival, migration and motility mediated through its specific G-protein-coupled receptors ($LPAR_{1-6}$). Blood LPA levels are risen is patients with chronic kidney disease of diverse aetiologies compared to healthy subjects, while it has been implicated in fibrosis at several other organs. Therefore, we wanted to investigate the effect of LPA on renal TECs. Hence, the normal kidney proximal TEC line HKC-8 was exposed to LPA and 174 other inflammatory-immunological stimuli. The levels of 27 intracellular phosphoproteins and 32 extracellular cytokines upon each one of the stimuli were measured employing custom multiplex ELISA. LPA stimulated the phosphorylation of JUN, IKBA, MKO3 (ERK1) and CREB1 and the expression of IL-6, IL-8, ICAM1, CCL2, CCL3, CXCL7. Stimuli were clustered according to their responses and, interestingly, LPA clustered together with proinflammatory molecules IL1A, IL1B, Tnf-a, Fsl1, PMA and promethazine. In order to verify the effects of LPA, cells were treated with three different species of LPA with similar results. Further verification of the induced cytokines was performed at the mRNA level with real time PCR. Conclusively, LPA emerges as a proinflammatory stimulus able to activate TECs, while several other stimuli are identified.

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P94

Studying the impact of mutations on the "linchpin" residue of Arkadia, an E3 ubiquitin ligase

Nefeli Fragkaki[1], Maria Birkou[1], Georgia N. Delegkou[1], Konstantinos D. Marousis[1], Vasso Episkopou[2], Georgios A. Spyroulias[1*]
[1]Department of Pharmacy, University of Patras, Patra, Greece
[2]School of Medicine, Imperial College, London, United Kingdom

Ubiquitination is a post-translational process for the smooth function of many organisms. Through ubiquitination pathway target proteins are labeled with ubiquitin (Ub) and prepared for degradation. Three enzymes are vital in this this pathway, E1 activating enzyme, E2 conjugating enzyme and E3 Ubiquitin ligase. Human Arkadia is an E3 RING-H2 Ub ligase consisting of 994 amino acids and acts as a positive regulator of the TGF-β pathway by catalyzing the degradation of Smad7, c-Ski and SnoN. It preferably interacts with the E2 enzyme UbcH5b[1] to efficiently transfer Ub to the target protein and it also has the ability of auto-ubiquitination. According to many studies[2,3], when RING E3s interact with E2-Ub conjugate, they promote a "closed" conformation. The mechanism behind this step is very complex and still not fully understood. It is presumed that this shift in the conformational equilibria, from "open" to "closed" conformation, is enhanced through a conserved residue, the allosteric "linchpin", of RING E3s that engages both the Ub and the E2 to restrict their relative orientations. According to our group's unpublished experimental data, Arkadia seems to lead the UbcH5b-Ub conjugate to a "closed" conformation. In this study, we examine the effect of two mutations on this "linchpin" Arginine 983, and specifically, the impact of the mutation of arginine to alanine and lysine. Arginine is one of the most common and efficient "linchpin" residues amongst the RING ligases due to its ability to form hydrogen bonds (multiple hydrogen bond donor groups) with ubiquitin and E2. Lysine is another "linchpin" residue that exists in some RING ligases and acts as hydrogen bond donor. Alanine on the other hand is a hydrophobic residue with a short aliphatic side-chain. The results of this study are expected to provide important insights considering the role of Arginine in the ubiquitination mechanism of Arkadia.

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P95

Oil-in-water microemulsions as carriers of compounds of pharmaceutical interest – Dermal applications

Ioanna Theochari[1], Tanja Ilic[2], Ines Nikolic[2], Vladimir Dobricic[2], Snezana Savic[2], Demetrios Papahatjis[1], Alia Tenchiu[1], Harris Pratsinis[3], Aristoteles Xenakis[1], Vassiliki Papadimitriou[1] and Vasiliki Pletsa[1]

[1]*National Hellenic Research Foundation, Institute of Chemical Biology, Athens, Greece*

[2]*University of Belgrade, Faculty of Pharmacy, Department of Pharmaceutical Technology and Cosmetology, Belgrade, Serbia*

[3] National Centre of Scientific Research "Demokritos", Institute of Biosciences and Applications, Athens, Greece

During the last decade, studies focusing on the development of biocompatible nanodispersions that can be delivered to the skin and treat severe skin disorders, including cancer, have been reported. In this context, an oil-in water (O/W) biocompatible microemulsion composed of PBS buffer, Tween 80, and triacetin was assessed as a drug carrier of DPS-2, a lead compound, initially designed in-house to inhibit $BRAF^{V600E}$ oncogenic kinase. DPS-2 is a pharmaceutical benzothiophene analogue exhibiting significant cytotoxicity towards various cell lines. The efficacy of the system was evaluated through both *in vitro* and *ex vivo* approaches.

The cytotoxic effect of both microemulsions and their cargo was examined through the MTT cell proliferation assay in melanoma cell lines and normal skin fibroblasts. The microemulsion, as carrier, had no effect on cell lines' viability while DPS-2 exhibited cytotoxicity in all cell lines, when loaded in microemulsions. The mechanism of the DPS-2-induced cell death was investigated through Fluorescence-activated cell sorting (FACS) analysis, Comet assay and Western Blotting of cell death markers. Overall, DPS-2 was effectively encapsulated and delivered within cells inducing a necrotic cell death through S-phase delay.

Porcine ear skin was used as a skin model *ex vivo* to evaluate the degree of permeation of DPS-2 through skin and assess its retention.The evaluation included permeation study through Franz cell device and differential tape stripping. It was clarified that encapsulated DPS-2 was distributed within the full thickness of the stratum corneum (SC) and had a high affinity to hair follicles. The developed O/W microemulsions enhanced the penetration of DPS-2 through skin layers, however, DPS-2 was retained in the skin which indicates that the system is only suitable for dermal and not transdermal delivery.

Overall, the evidence generated paves the way for the development of biocompatible nanoformulations for targeted delivery of lipophilic anticancer drugs against skin cancer.

Keywords: nanodispersions, chemotherapy, encapsulation, *ex vivo* permeation, cell viability

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P96

Investigating the role of RANKL in breast cancer and bone metastasis

Evi Gkikopoulou[1, 2], Anthi Kolokotroni[1, 2], Vagelis Rinotas[2], Melina Dragolia[2], Vasileios Ntafis[2], Martina Rauner[3], Eleni Douni[1, 2,]

[1] *Department of Biotechnology, Agricultural University of Athens, Iera Odos 75, 11855, Athens, Greece*

[2] *Institute for Bioinnovation, B.S.R.C. "Alexander Fleming", Fleming 34, 16672, Vari, Greece*

[3] *Department of Medicine III, Faculty of Medicine, Dresden, Germany*

Receptor activator of nuclear factor-κB ligand (RANKL), a member of TNF-a superfamily, is known for its crucial role in bone remodeling. Recently, RANKL has been associated with the development of breast tumors, promotion of cancer cell migration and invasion and, also, the formation of subsequent bone metastases. In the present study we investigated the function of RANKL in breast cancer and skeletal metastasis, using transgenic mice that overexpress human RANKL (TgRANKL). Our breast cancer model was generated by injecting E0771 breast cancer cells orthotopically in the abdominal mammary glands of the mice. Since E0771 cell line is stably transduced with the firefly luciferase gene, we were able to monitor breast cancer initiation and progression through *in vivo* bioluminescence imaging. Even though mammary tumors were detected both in TgRANKL mice and their wild-type (WT) littermates, we noticed larger tumors in our transgenic mice, although, histological examination did not reveal any differences between the two groups. Except for breast cancer, we additionally established a skeletal metastasis model by injecting E0771 cells into the tibiae of WT and TgRANKL mice. Tumor lesions were detected by both X-ray radiation and bioluminescence imaging, whereas upon isolation, tumor-bearing bones were analyzed through micro computed tomography (micro-CT) and histological analysis. In conclusion, we have established a novel RANKL-dependent *in vivo* model for breast cancer and bone metastasis related studies. Our upcoming goal is to perform both *in vitro* and *in vivo* experiments to further elucidate the mechanisms underlying the function of RANKL in breast cancer and cancer-initiated metastasis.

P97

Biglycan and WNT signaling pathways interact to regulate MG63 osteosarcoma cells growth

Eirini Maria Giatagana[1], Aikaterini Berdiaki [1], John Aggelidakis[1], Dragana Nikitovic[1]
[1]Laboratory of Histology-Embryology, School of Medicine, University of Crete, Heraklion, Greece

Osteosarcoma is a malignant tumor characterized by the production of an abundant extracellular matrix (ECM). Proteoglycans, structural components of the bone ECM, or osteoid, modulate signaling pathways involved in specific bone pathogenic phenotypes. WNT signaling pathway is known to participate in bone formation. We have recently shown that biglycan, a small leucine-rich proteoglycan (SLRP), is a positive regulator of osteosarcoma cell growth through an insulin-like growth factor receptor I (IGF-IR)/LRP6/b-catenin signaling axis. Treatment of MG63 cells with recombinant biglycan leads to increased b-catenin expression and deposition to the cytoplasm and nucleus. Silencing biglycan expression of MG63 cells with specific siRNAs results in an attenuated transcription of the b-catenin target gene, cyclin D1. Immunofluorescence showed that biglycan treatment enhanced its own co-localization with LRP6, a WNT co-receptor, and b-catenin/pIGF-IR co-localization. When MG63 cells were exposed to an IGF-IR inhibitor, AG1024, a decreased b-catenin deposition to the nucleus was determined. Previous experiments have shown that targeting biglycan expression with siRNAs specific for biglycan also decreased the phosphorylation of ERK, a mediator of IGF-IR receptor signaling. The deregulation of these signaling pathways is associated with loss of function or decreased expression of the PTEN, a tumor suppressor gene. Treatment of MG63 cells with biglycan leads to reduced expression of PTEN and the inhibition of GSK-3b, a kinase that regulates b-catenin activation. Furthermore, treated with biglycan MG63 cells express higher levels of tumor aggressiveness markers, vimentin, and fibronectin compared to controls. Our present results demonstrate that biglycan enhances WNT signaling pathway activation and the interaction of b-catenin with the activated IGF-IR in MG63 osteosarcoma cells, promoting cell aggressiveness and proliferation. Targeting this mechanism could be a key to improving the existing treatments and enhancing osteosarcoma chemosensitivity.

P98

ESR2 drives epithelial-to-mesenchymal-transition and tumor growth through epigenetic signatures in aggressive breast cancer

Zoi Piperigkou[1,2*], Vasiliki Zolota[3], Marco Franchi[4], Martin Götte[5], Nikos Karamanos[1,2]
[1]Biochemistry, Biochemical Analysis & Matrix Pathobiology Research Group, Laboratory of Biochemistry, Department of Chemistry, University of Patras, Greece
[2]Foundation for Research and Technology-Hellas (FORTH)/Institute of Chemical Engineering Sciences (ICE-HT), Patras, Greece
[3]Department of Pathology, School of Medicine, University of Patras, Greece
[4]Department for Life Quality Study, University of Bologna, Rimini, Italy
[5]Department of Gynecology and Obstetrics, University Hospital Münster, Germany

Estrogens and their receptors (have pivotal roles in the development and progression of breast cancer. It is well established that interactions among cancer cells and tumor microenvironment are in dynamic interplay and regulated by extracellular matrix (ECM). We have recently revealed that *ERβ* suppression in MDA-MB-231 breast cancer cells reduces their aggressive phenotype through the inhibition of epithelial-to-mesenchymal transition (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 ERβ suppression following clone selection in breast cancer cells transfected with shRNA against human *ERβ* (*ESR2*) that resulted in 90% reduction of ERβ mRNA and protein levels. We demonstrated that ERβ 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 ERβ targeting in future pharmaceutical approaches for managing the aggressive breast cancer.

P99

A CRISPR/Cas9-induced mutant zebrafish line reveals the essential role of *cfdp1* in cardiac development and function

Panagiota Giardoglou[1,2], Panos Deloukas[3], George Dedoussis[2], Dimitris Beis[1*]
[1] *Center for Clinical, Experimental Surgery & Translational Research, Zebrafish Disease Model Lab, Biomedical Research Foundation, Academy of Athens, Athens, Greece*
[2] *Department of Nutrition and Dietetics, School of Health Science and Education, Harokopio University of Athens, Athens, Greece*
[3] *Clinical Pharmacology, William Harvey Research Institute, Barts and The London Medical School, Queen Mary University of London, London, UK*

Cardiovascular diseases (CVD) describe disorders related to the morphology and function of the heart and blood vessels. CVDs are the main cause of mortality worldwide and the risk factors associated with CVD traits are not only environmental but also genetic. Thus, it's highly important to identify candidate genes and elucidate their role in the manifest of the disease. Large-scale human studies have revealed the implication of Craniofacial Development Protein 1 (*CFDP1)* in coronary artery disease (CAD). *CFDP1* belongs to the evolutionary conserved Bucentaur (BCNT) family and up to date, its function and mechanism of action in Cardiovascular Development is still unclear. In this study, we utilize zebrafish to investigate the role of *cfdp1* in the developing heart due to the high genomic homology, similarity in heart physiology and the ease of experimentally manipulation. We showed that *cfdp1* is expressed during development and at 120 hours post fertilization its expression is restricted to the region of the heart and the head. We then generated a *cfdp1*-null zebrafish line using CRISPR-Cas9 system which led to a lethal phenotype since *knockout* embryos do not reach adulthood. *cfdp1*$^{-/-}$ embryos develop arrhythmic hearts and cardiac dysfunction exhibiting statistically significant differences in heart features including End Diastolic Volume, Cardiac Output, Ejection Fraction and Stroke Volume. Myocardial trabeculation is also impaired in *cfdp1*$^{-/-}$ embryonic hearts, implying its regulatory role also in this developmental process. Findings from both *knockdown* and *knockout* experiments showed that abrogation of *cfdp1* leads to downregulation of Wnt signaling in embryonic hearts during valve development but without affecting Notch activation in this process. We will present the phenotypic characterization of this mutant line.

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P100

Intestine-specific ablation of the Hepatocyte Nuclear Factor 4a (*Hnf4a*) gene in mice has minimal impact on serum lipids and ileum gene expression profile due to upregulation of its paralog *Hnf4g*

Efstathia Thymiakou[1,2], Efsevia Xenikaki[1] and Dimitris Kardassis[1,2*]
[1]*Laboratory of Biochemistry, University of Crete Medical School, Heraklion 71003, Greece*
[2]*Gene Regulation and Genomics group, Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology of Hellas, Heraklion 70013, Greece*
**kardasis@imbb.forth.gr*

Our aim was to investigate the role of Hepatocyte Nuclear Factor 4A (HNF4A) in lipoprotein metabolism using mice with genetic ablation of *Hnf4a* specifically in the intestine. H4IntKO mice presented normal serum lipid levels, HDL-C and HDL particle size (α1-α3). RNA-seq analysis (Illumina) revealed that in the ileum of H4IntKO mice 128 differentially expressed transcripts were downregulated and 217 genes were upregulated and identified metabolic pathways significantly affected by *Hnf4a* ablation such as type II diabetes, glycolysis, gluconeogenesis and p53 signaling. The expression of the major HDL biogenesis genes *Apoa1*, *Abca1* and *Lcat* was not affected but there was significant increase in *Apoc3* as well as in *Hnf4g*, a paralog of *Hnf4a*. Interestingly, *Hnf4g* expression was induced both at mRNA and protein level in the ileum of H4IntKO mice. In HEK293T cells, HNF4G2 isoform activated the promoters of *Apoc3*, *Apoa4*, *Abcg5* and *Abcg8* to similar or higher levels than HNF4A. Chromatin immunoprecipitation assays showed that HNF4G bound to various apolipoprotein gene promoters in control mice but its binding affinity was reduced in the ileum of H4IntKO mice suggesting a redundancy but also a cooperation between the two factors. In the distal colon of H4IntKO mice, where both HNF4A and HNF4G are absent and in a mouse model of DSS-induced colitis presenting decreased levels of HNF4A, most lipoprotein genes were strongly downregulated. In conclusion, *Hnf4a* ablation in mice does not significantly affect serum lipid levels or lipoprotein gene expression in ileum possibly due to compensatory effects by its paralog *Hnf4g* in this tissue.

P101

Evaluation of Heat Shock Protein 70 in inflammatory pain in vivo

Maria Minadaki, Michail Deiktakis, Aikaterini Kalantidou, Eirini Vassilakaki and Maria Venihaki*
Laboratory of Clinical Chemistry, Medical School, University of Crete, Heraklion, Crete, Greece

Introduction
Heat Shock Protein 70 (HSP70) belongs to a family of Heat Shock Proteins that, intracellular, are catalytically involved in the protein folding process and play an important role in maintaining cellular homeostasis. In addition, they participate in cell transportation and signal transduction processes.
During inflammation, HSP70 protein levels are elevated, providing cytoprotection and participating in immunoregulation. In particular, HSP70 protein has been shown to promote the production of anti-inflammatory cytokines and significantly inhibit inflammatory signaling pathways.
Inflammatory pain is the spontaneous hypersensitivity to noxious stimuli such as tissue damage or infection and is accompanied by activation of the immune system.
Based on the above, **the aim of this study** was the investigation of the effect of HSP70 deficiency on inflammatory pain and the subsequent activation of the immune system.
Methods
Wild-type mice (WT) and mice with HSP70 protein gene deficiency (HSP70 KO) were used. Inflammation was induced by intraplantar injection of 20 μl CFA (Complete Freund's adjuvant). Pain threshold was measured 3, 6 and 24 hours after the injection with the use of the Hargreaves Apparatus. Paw oedema was also evaluated at the same time points by a plethysmometer. At the end of the experiments, mice sacrificed and blood and tissues were collected for further analysis. Proinflammatory cytokines were measured with Elisa. NGF and PENK mRNA were evaluated by Real-time PCR.
Results - Discussion
HSP70-deficient mice have significantly lower pain threshold after the induction of inflammation. Surprisingly, they had less oedema compared to wild-type mice. Deficiency of HSP70 was accompanied by increased concentration of spleen proinflammatory cytokines and higher paw NGF mRNA levels, suggesting that HSP70 deficiency is associated with less inflammation. Further studies are underway to identify the possible mechanism by which HSP70 exerts its effects.

P102

Expression, purification and evaluation of the antigenic properties of West Nile virus Recombinant Capsid protein produced in E.coli and Insect Sf9 cells

Eftychia Filippopoulou[1], Constantine Meliopoulos[1], Theodora Mantso[1], Vasilis Xanthis[1], Vasiliki E. Fadouloglou[1], Ioannis Karakasiliotis[2], Katerina R. Katsani[1], Penelope Mavromara[1]
[1]Department of Molecular Biology & Genetics, Democritus University of Thrace, Alexandroupolis, Greece
[2]Department of Medicine, Democritus University of Thrace, Alexandroupolis, Greece

West Nile virus (WNV) is a mosquito-borne pathogen that may cause neuroinvasive disease, which can lead to fatal meningoencephalitis. WNV is a member of the *Flaviviridae* family. The viral genome is a positive-sense, single-strand RNA molecule of about 11 kb in size and encodes 7 non-structural and 3 structural proteins, the capsid (C), the pre-membrane (prM), and the envelope (E) protein. These three proteins participate in the formation of the enveloped, icosahedral viral capsid.

The E protein is the major antigenic site for neutralizing antibodies and is often used alone or in combination with prM -in the form of subviral particles (SPs)- as antigen for the development of WNV serological assays. These tests, however, are hampered by cross-reactivity among other flaviviruses[1] and the development of new WNV serological tests with improved specificity is required. On the other hand, little is known on the antigenicity of the C protein.

In the present study, we produced recombinant C protein using both a prokaryotic and a eukaryotic expression system. We cloned the cDNA coding the C protein from West Nile virus strain NY99 in the pET-28a(+) vector and expressed it in the Rosetta (DE3) pLysS *E. coli* strain. We also cloned the same cDNA in the HpFastBac vector and expressed it in *Sf9* insect cells, using the Bac-to-Bac baculovirus expression system. As both expression vectors are designed to introduce a His-tag to the cloned sequence, we were able to confirm the expression in both systems, via Western blot, using an anti-His tag antibody. Currently, we are optimizing the conditions for the purification of the recombinant protein produced in both expression systems using Ni-NTA affinity chromatography. Our long-term objective is to develop and evaluate home-made serological assays based on these recombinant proteins, in order to assess the prevalence of WNV infection in Thrace.

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P103

Conformational Study of the RNA-binding *human* La (Lupus antigen) protein through NMR Spectroscopy

Georgios A. Machaliotis[1], Aikaterini Argyriou[1], Eleni Kaliatsi[2], Constantinos Stathopoulos[2], Georgios A. Spyroulias*[1]

[1] *Department of Pharmacy, University of Patras, GR-26504, Patras, Greece*

[2] *Department of Biochemistry, School of Medicine, University of Patras, GR-26504, Patras, Greece.*

**Corresponding author*

Lupus antigen (La) protein, is an RNA-binding protein, ubiquitous in human cells, shuttling between the nucleus and the cytoplasm and interacting with different RNAs. La is mainly located in the nucleus, associates with all RNA polymerase III (pol III) transcripts and modulates their maturation processing, being the first factor to interact with these transcripts. There is reported evidence that La affects the translation of some cellular and viral mRNAs in the cytoplasm. Although there are no structural data available so far, La function depends on the cooperation of its four domains that comprise the protein. More specifically, it consists of an La motif (LaM) and an RNA Recognition Motif (RRM) at the N- terminal domain of the protein and a second RRM and a C-terminal region at the C-terminal domain of the protein.

Through NMR (Nuclear Magnetic Resonance) Spectroscopy we investigate the structure and the dynamical properties of La protein in solution. We studied two different polypeptides, the N- terminal domain, LaM-RRM1 (194aa) and the C-terminal domain of the protein, RRM2α including the C-terminal region (185aa) and we report a sequence-specific assignment of their ^{1}H, ^{15}N, and ^{13}C resonances. Based on these assignment data, we obtained the secondary structure elements for both constructs using TALOS+ server. Through relaxation experiments, we gain information about the plasticity and flexibility of the protein in its free form.

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P104

Ectopic overexpression of T cell kinase Lck triggers B cell receptor (BCR) signaling independent of receptor ligation

Kyriakos Konnaris[1*], Vasileios Morfos[1] and Konstantina Nika[1]
[1]Department of Biochemistry, School of Medicine, University of Patras, Patras, Greece

CLL cell survival and expansion depends on unremitting signals from the B-Cell Receptor (BCR). Several groups, including ours, have documented the concurrence between ectopically expressed Lck in CLL B cells, and enhanced BCR signaling. Further to that, our previous work implied that high levels of Lck may trigger signaling responses independently of receptor ligation (autonomous BCR signaling). However, affirmation of a cause-effect relationship is still missing.
To establish a direct correlation between ectopic Lck, and triggering of BCR signaling pathways, we developed a model B cell line in which we introduced the Lck gene via lentiviral gene transfer using a Tet-On inducible system. To obtain a global image of the impact of Src family kinase (SFK) overexpression on BCR signaling, we also established stable line overexpressing Lyn, which is the physiological "resident SFK" in B cells. Our results showed that both Lck and Lyn overexpression induce constitutive phosphorylation of BCR ITAMs and downstream signaling mediators such as Syk and Akt in the absence of receptor ligation. Signaling responses following BCR stimulation also appeared to be more robust in the presence of Lck and Lyn.

P105

Differential DNA methylation in lymphoma subtypes sheds light on their heterogeneity

Angelos Galanopoulos[1,2], Georgia Orfanoudaki[2,3], Alexandros Triantafyllidis[1], Konstantina Psatha[2,3,4], Michalis Aivaliotis[*2,3,4]

[1]Department of Genetics, Development and Molecular Biology, School of Biology, Aristotle University of Thessaloniki, Thessaloniki, Greece

[2]Functional Proteomics and Systems Biology (FunPAth), Center for Interdisciplinary Research and Innovation (CIRI-AUTH), Thessaloniki, Greece

[3]Institute of Molecular Biology and Biotechnology, FORTH, Heraklion, Greece

[4]Laboratory of Biological Chemistry, School of Health Sciences, Department of Medicine, Aristotle University of Thessaloniki, Thessaloniki, Greece

[*]Correspondence: aivaliotis@auth.gr

DNA methylation is an essential epigenetic mechanism determining gene expression regulation and cell differentiation, but its abnormal dysregulation may have substantial consequences, e.g. cancer development1. Lymphomas are a group of blood malignancies that develop from B, T/NK cells, featuring a wide range of heterogeneous subtypes2. Diagnosing and treating lymphomas is often difficult, due to lack of knowledge on their molecular pathobiology. The goal of this study was the identification of genes with significant differential DNA methylation in Diffused Large B-cell Lympoma's (DLBCL) subtypes vs. healthy controls. Towards this end, we analyzed a publicly available dataset from the EBI (European Bioinformatics Institute) database using the R programming language and employing packages from the Bioconductor platform3. An existing workflow was employed for the analysis of the methylation data, with certain modifications to fit the dataset. The steps of the analysis were: a) data preprocessing, including quality control, normalization, filtering, b) identification of statistically significant differentially methylated CpGs and genomic regions, and c) gene set enrichment analysis using terms/pathways from the databases Gene Ontology (GO)4, Kyoto Encyclopedia for Genes and Genomes (KEGG)5 and MSigDB6. The differentially methylated CpGs were calculated for each comparison and, based on that, the differentially methylated regions were identified. The genes overlapping with the most statistically significant differentially methylated regions were further investigated. 14 genes were identified to be differentially methylated (IRX3, UNCX3, GATA4, MYOD1, EOMES, GATA3, NKX6-2, FOXG1, FAM46A, AP2A2, COL11A2, EDNRB, COL25A1, EN1). Most of them encode transcription factors and demonstrated significant methylation in ABC and GC DLBCL, but not in gastric DLBCL and healthy controls. Concluding, our study demonstrated that DNA methylation differs significantly between lymphoma patients and healthy controls, and between lymphoma subtypes. Such results on DNA methylation, an important indicator of gene expression, may contribute to the understanding of lymphoma subtypes heterogeneity and molecular pathology.

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P106

Development of blocking Nanobodies as potential Lck inhibitors

Ioannis Tyritidis[1*], Kyriakos Konnaris[1], Nikos Koutras[1], Vasilis Morfos[1], Konstantina Nika[1]
[1]Department of Biochemistry, School of Medicine, University of Patras, Patras, Greece

Deviant T lymphocyte responses are causative for a large number of pathological conditions. Hence intense efforts are focused on identifying compounds capable of manipulating signaling pathways leading to T cell activation. The Lymphocyte-specific protein tyrosine kinase (Lck), a member of the Src family of protein tyrosine kinases (SFKs), is absolutely mandatory for T cell function and has become a very attractive target for the production of small molecule inhibitors. Despite tremendous efforts, the development of highly selective and potent Lck inhibitors, suitable for clinical use, has not met with success, due to an astonishing structural homology shared by SFK members within their catalytic centers. Since SFKs are expressed in almost all cell types and are vital for a plethora of biological functions, limited selectivity of Lck inhibitors has been reported to result in off-target toxicity. The purpose of this project is to assess a novel approach of Lck inhibition with a desired specificity. To achieve this, we have generated innovative nanobodies (Nbs), targeting a poorly conserved region of Lck. These Nbs have been modified by the addition of an Endoplasmatic Reticulum (ER) retention signal, aiming to reduce the expression levels of newly synthesized Lck.
An initial screen of different Nbs was accomplished by transfecting HEK293T cells with Lck in presence or absence of Nbs. Lck expression levels were quantitated by Western Blot and FACS, whereas the ability of Nbs to bind Lck was assessed by Co-Immunoprecipitation experiments.
Our data showed that at least 3 Nbs were capable of binding Lck and significantly reduce its levels. These findings support our notion that Nbs produced in our lab could be effective inhibitors of Lck. Our next goals are to test whether the Nbs are exclusively specific for Lck and to elucidate the function of Nbs in primary human T lymphocytes.

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P107

Aberrant signaling leading to translation rewiring in two different melanoma cell lines with acquired resistance to a BRAF inhibitor

Argyris Alexiou[1], Vassilis Stamatakis[1], Antonia Petropoulou[1], George Kyriakopoulos[1], Katerina Grafanaki[1,2] and Constantinos Stathopoulos[1*]

[1] *Department of Biochemistry, School of Medicine, University of Patras, Greece*

[2] *Department of Dermatology, School of Medicine, University of Patras, Greece*

Melanoma is a highly aggressive and metastatic type of cancer which is mainly driven by the $BRAF^{V600E}$ mutation in more than 50% cases. $BRAF^{V600E}$ mutation leads to aberrant downstream signaling through the MAPK cascade and affects transcription and translation rates. Although specific small-molecule BRAF inhibitors, like vemurafenib, are currently in clinical use to restrain aberrant signaling, the quick adaptation of melanoma cells to the therapy results in aggressive relapse. The complex molecular mechanisms that lead to vemurafenib resistance (VR) affect translation initiation factors and rates leading to an overall metabolic rewiring. To get new insights on the translational reprogramming of resistant cells, A375 (primary) and SK-MEL-5 (metastatic) melanoma cell lines were cultured with increased concentrations of vemurafenib until they acquired resistance at 3 μM. Subsequent analysis of the mRNA and protein levels of both resistant and sensitive cell lines showed that the two VR cell lines follow different pathways for the acquisition of resistance. A375 VR showed reduced expression and activation of factors that favor cap-dependent translation, such as members of the eIF4F complex, but also a significant hypophosphorylation of S6 kinase, an mTORC1 substrate, and lower global protein synthesis rates. In contrast, the metastatic Sk-Mel-5 VR cell line showed elevated phosphorylation of the S6 kinase and activation of factors that relate to cap-dependent translation. Additionally, puromycin staining and polysome profiling analysis indicated the upregulation of global translation rates in Sk-Mel-5 VR cells. Collectively, our results suggest that acquired resistance can lead to the differential rewiring of translation initiation reflecting the high adaptability of melanoma cells in cancer treatment.

P108

Coriander heptenol induces aberrant gravitropism response in *Arabidopsis thaliana* primary root

Dimitris Pappas[1], Ioannis-Dimosthenis S. Adamakis[2], Spyros Gkelis[1], Triantafyllos Kaloudis[3], Emmanuel Panteris[1]*

[1] *Department of Botany, School of Biology, Aristotle University of Thessaloniki, Greece*
[2] *Section of Botany, Faculty of Biology, National and Kapodistrian University of Athens, Greece*
[3] *Institute of Nanoscience and Nanotechnology, NCSR Demokritos, Athens, Greece*
* *correspondence: epanter@bio.auth.gr*

Coriander heptenol (6-methyl-5-hepten-2-ol) is an odorous volatile organic compound (VOC) produced by coriander (*Coriandrum sativum*) and various microorganisms, including many cyanobacterial strains. Since cyanobacteria thrive in freshwater, where they can form extensive blooms, the occurrence of coriander heptenol in water destined for irrigation is likely, thus becoming accessible to crops. Although bacterial VOCs have been associated with diverse effects on plant growth, no published data exist regarding the effects of coriander heptenol on plants. In this study, the effects of coriander heptenol on the primary root of the model plant *Arabidopsis thaliana* were investigated. Four-day-old wild type (ecotype Columbia) seedlings, as well as transgenic lines expressing the auxin response reporter DR5:GFP or GFP-tagged variants of the PIN-FORMED (PIN) auxin efflux carriers, particularly PIN1, PIN2 and PIN3, were treated with various concentrations of coriander heptenol. Untreated and VOC-treated roots were observed with light and epi-fluorescence microscopy, while some specimens were prepared for Transmission Electron Microscopy (TEM) or underwent tubulin immunolabelling and were examined with a Confocal Laser Scanning Microscope (CLSM). Root tips of seedlings exposed to high concentrations of coriander heptenol exhibited deviated growth, resulting in "hook-like" configuration. Although cortical microtubules and cell ultrastructure in the bending root region appeared unaffected, PIN distribution was altered. Furthermore, gravitropism experiments revealed that roots treated with lower VOC concentrations exhibited delayed gravitropic response, compared to untreated ones. These results suggest that coriander heptenol disrupts auxin transport in *Arabidopsis thaliana* roots. Investigation of auxin-related gene expression could offer further information on the above effect.

P109

Comperative study of bacterial homologs of β1 H-NOX domain of human soluble guanylyl cyclase (sGC)

Styliani A. Chasapi[1], Garyfallia I. Makrynitsa[1], Aikaterini I. Argyriou[1], Aikaterini A. Zompra[1], Georgios A. Spyroulias[1*]
[1]Department of Pharmacy, University of Patras, Patras, Greece
**e-mail: G.A.Spyroulias@upatras.gr*

Soluble guanylate cyclase (sGC) is considered as the primary NO receptor across several known eukaryotes. The main interest regarding the biological role and its function, focuses on the H-NOX domain of the β1 subunit. This domain in its active form bears a ferrous b type heme as prosthetic group, which facilitates the binding of NO and other diatomic gases. The key point that still needs to be answered is how the redox state of heme determines H-NOX active state and coordination upon binding of diatomic gases. H-NOX domain is present in the genomes of both prokaryotes and eukaryotes, either as a stand-alone protein domain or as a partner of a larger polypeptide. The biological functions of these signaling modules for a wide range of genomes, diverge considerably along with their ligand binding properties [1]. In this direction, we examine five prokaryotic H-NOX proteins from *Nostoc punctiforme* (*Npun* H-NOX), *Vibrio Cholerae* (*Vb* H-NOX), *Caldanaerobacter subterraneus* (*Cs* H-NOX), *Shewanella oneidensis* (*So* H-NOX) and *Shewanella woodyi* (*Sw* H-NOX). These domains share some common amino acids in their sequence that may explain to an extent the organism-specific ligand preference. Since Fe-heme as a substrate can bind diatomic gases with binding affinities of the order NO>>CO>>O_2, we aim to address the perception of the redox state switch mechanism of heme [2]. Based on physicochemical and spectroscopical properties we intend to highlight the significant structural induced alterations in common key-regions of the selected H-NOX domains.

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P110

Cooperation of sumoylation with hypoxia for transcriptional regulation: The role of TFAP2A

Amalia Kanoura[1], Antonis Giakountis[2], Chrysa Filippopoulou[1], George Stamatakis[3], Martina Samiotaki[3], George Panayotou[3], George Simos[1] and Georgia Chachami[1]*,
[1]*Laboratory of Biochemistry, Faculty of Medicine, University of Thessaly, Biopolis 41500, Larissa, Greece,*
[2]*Department of Biochemistry and Biotechnology, University of Thessaly, Larissa 41500, Greece*
[3] *Institute for Bioinnovation, Biomedical Sciences Research Center "Alexander Fleming", 16672, Vari, Attica, Greece*
**email: ghah@med.uth.gr*

Hypoxia, a state of oxygen deficiency, occurs in pathological conditions such as cancer due to the imbalance between oxygen supply and consumption. When oxygen becomes limited, the hypoxic signalling pathway enables maintenance of homeostasis and adaptation of cells and tissues. The main molecular regulators of this pathway are the Hypoxia Inducible transcription Factors (HIFs). Post-translational modifications (PTMs) have a major effect on hypoxic adaptation and on HIF activity. Sumoylation is the covalent attachment of Small Ubiquitin-related MOdifier (SUMO) to proteins. Sumoylation of specific target proteins or of group of proteins occurs under stressful conditions such as ischemia or hypoxia, which overall facilitates cell adaptation and survival. Recent data from quantitative proteomic analysis of endogenous sumoylated proteins led to the identification of several proteins, mainly transcription factors and regulators, that exhibit altered sumoylation status under hypoxia[1]. One of these proteins is TFAP2A (Transcriptional Factor Activating enhancer binding Protein 2 alpha), a protein that is critical for the regulation of gene expression of many genes, especially during early stages of development, as well as oncogenesis and tumor progression. We have shown that sumoylation of TFAP2A is reduced under hypoxia. In addition, TFAP2A physically interacts with HIF-1α and its sumoylation status affects HIF transcriptional activity. The involvement of TFAP2A in HIF-dependent gene regulation is currently under investigation using ChIP-Seq approaches. Using overexpression of mutant TFAP2A constructs that mimic or inhibit its sumoylation and IP-proteomic techniques, we are also analysing the protein interaction networks between TFAP2A and HIFs and their involvement in the cellular adaptation to hypoxia. As both TFAP2A and HIFs are critical players in oncogenesis, understanding their interrelated pathways can be valuable for the development of effective molecular therapeutic interventions.

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P111

Serrapeptase as an antibiofilm agent and its effect on microbial viability, phosphorolytic activity, and virulence factors

Georgios Katsipis [1], Anastasia A. Pantazaki [1]
[1]*Laboratory of Biochemistry, Department of Chemistry, Aristotle University of Thessaloniki, 54124, Thessaloniki, Greece*

Biofilms are three-dimensional bacterial communities that are formed on biotic or abiotic environments, embedded within an extracellular matrix of peptidoglycans and amyloids (Congo-red-positive staining). Biofilms are characterized by increased resistance against antibiotics and the host immune system, and their presence ignites a persistent inflammation, found in most chronic infections. Previous studies in biofilm formation indicate the importance of alkaline phosphatase(s) (ALP), as well as of bacterial virulence factors (lipopolysaccharides – LPSs, flagellin, and lipoteichoic acid – LPT). Serrapeptase (SPT) is a serine-protease firstly isolated from silkworm bacteria *Serratia marcescens*. Studies have indicated that SPT is a promising enzyme for therapeutic opportunities, as it was found to possess anti-inflammatory and anti-microbial properties, which often surpass the ones of antibiotics, as well as anti-amyloidogenic activities. Though several studies prove the efficacy of SPT against biofilm formation, the exact mechanism is not yet elucidated. In this study, the antibiofilm activity of SPT has been demonstrated in Gram-negative bacterium *Escherichia coli* (ATCC25922), and Gram-positive bacteria (ATCC25923) and Methicillin-Resistant *Staphylococcus aureus* (MRSA). Similar activity was not observed by trypsin or albumin. Additionally, SPT slightly suppresses the growth and viability of the studied bacteria, and in a positive correlation with biofilm inhibition of *S. aureus*. Moreover, SPT-treated *S. aureus* presents reduced amyloids (Congo-red staining), and this effect correlates positively with biofilm inhibition. Activity of bacterial ALP has been found reduced in SPT-treated *S. aureus* and MRSA, and in correlation with inhibition of biofilm formation. On the contrary, ALP of *E. coli* was increased in SPT-treated bacteria. The effect of SPT treatment on LPS, flagellin and LPT levels of bacteria, has also been determined. These results prove the SPT potentiality for antibiofilm activity. Additionally, this study underlines the involvement of ALP for biofilm formation in Gram-positive bacteria, providing further approaches for confronting chronic infections.

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Evaluation of heat shock protein family B members HSPB2 and HSPB3 in bladder cancer prognosis and progression

Despoina D. Gianniou[1], Aimilia D. Sklirou[1], Margaritis Avgeris[2], Andreas Scorilas[2*], Ioannis P. Trougakos[1*]

[1]*Department of Cell Biology and Biophysics, Faculty of Biology, National and Kapodistrian University of Athens, Athens, Greece.*

[2]*Department of Biochemistry and Molecular Biology, Faculty of Biology, National and Kapodistrian University of Athens, Athens, Greece*

**e-mail: ascorilas@biol.uoa.gr*

itrougakos@biol.uoa.gr

Bladder cancer (BlCa) remains the fourth more commonly diagnosed type of male malignancy. Due to clinical heterogeneity of BlCa, novel markers will optimize treatment efficacy and improve the disease prognosis and management. The small heat shock protein (sHSP) family is one of the major groups of molecular chaperons. Some of their functions include the maintenance of proteome functionality and stability by proper protein folding and unfolding. However, the role of sHSPs in BlCa has not been thoroughly investigated yet. In this study, we investigated *HSPB2* and *HSPB3* mRNA expression in BlCa and their potential prognostic significance. For this purpose, we isolated total RNA from 80 bladder tumor samples and 56 paired non-cancerous bladder specimens, and developed an accurate SYBR-Green based real-time quantitative polymerase chain reaction (qPCR) protocol to quantify *HSPB2* and *HSPB3* mRNA levels in the two cohorts of specimens. For qPCR calculations, we applied the comparative Ct method for relative quantification; the housekeeping gene encoding hypoxanthine phosphoribosyltransferase 1 (*HPRT1*) served as reference gene, and BJ human newborn foreskin fibroblasts, were used as calibrator. Comparison of *HSPB2* and *HSPB3* mRNA levels uncovered the upregulation of mRNA expression in muscle-invasive (T2-T4) compared to superficial tumors (TaT1), and in high grade compared to low grade tumors. Next, we performed extensive biostatistical analysis including Kaplan-Meier survival analysis. Low *HSPB2* and *HSPB3* mRNA expression predicts poor disease-free survival (DFS) in TaT1 BlCa patients (*P*=0.008 and P=0.047, respectively). Thus, TaT1 patients with tumors poorly expressing *HSPB2* and *HSPB3* mRNA are more likely to relapse than others. In conclusion, *HSPB2* and *HSPB3* genes transcription is heavily upregulated in BlCa, compared to the normal tissue. Most importantly, low *HSPB2* and HSPB3 mRNA expression is associated with BlCa recurrence.

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NR5A2 as a potential drug target in Glioblastoma

Dimitrios Gkikas[1], Dimitris Stellas[2], Alexia Polissidis[3], Theodora Manolakou[1], Maroula Kokotou[3], George Kokotos[3] and Panagiotis K. Politis[1]

[1]Center for Basic Research, Biomedical Research Foundation of the Academy of Athens, 4 Soranou Efesiou Str., 115 27, Athens, Greece

[2]Institute of Chemical Biology, National Hellenic Research Foundation, 48 Vassileos Constantinou Ave, 116 35, Athens, Greece

[3]Centre for Clinical, Experimental Surgery & Translational Research, Biomedical Research Foundation of the Academy of Athens, 11527 Athens, Greece

[4]Center of Excellence for Drug Design and Discovery, and Department of Chemistry, National and Kapodistrian University of Athens, Panepistimiopolis, Athens 15771, Greece

Glioblastoma is characterized by rapid progression and poor survival rate. It is the most aggressive nervous system malignancy and despite recent advances in the provided therapy the average survival time remains extremely low, between 12 to 15 months. These clinical observations underscore the need for novel therapeutic insights and pharmacological targets. To this end, here we identify the orphan nuclear receptor NR5A2/LRH1 as a negative regulator of cancer cell proliferation and promising pharmacological target for nervous system-related tumors. In particular, by meta-analysing clinical data from TCGA and Oncomine databases, we find that high expression levels of NR5A2 are associated with favourable prognosis in patients with glioblastoma tumors. In addition, we experimentally show that NR5A2 is sufficient to strongly suppress proliferation of both human and mouse glioblastoma cells (U87-MG and GL261). The anti-proliferative effect of NR5A2 is mediated by the transcriptional induction of negative regulators of cell cycle, *CDKN1B* ($p27^{kip1}$), *CDKN1A* ($p21^{cip1}$) and *Prox1*. Consistently, silencing of NR5A2 induces proliferation and suppresses these genes. Interestingly, two well-established pharmacological agonists of NR5A2, DLPC and DUPC, are able to mimic the anti-proliferative action of NR5A2 in human glioblastoma cells. Most importantly, treatment with DLPC inhibits glioblastoma tumor growth *in vivo*, in heterotopic and orthotopic xenograft mouse models. These data indicate a tumor suppressor role of NR5A2 in nervous system and render this nuclear receptor as a potential pharmacological target for the treatment of nervous tissue related tumors.

P114

The improvement of glycemic and lipidemic profile induced by power training is mediated by alterations in the skeletal muscle fiber type composition

Spyridon Methenitis[1], **Tzortzis Nomikos**[2,*], **Thomas Mpampoulis**[1], **Eleni Kontou**[1], **Clio-Maria Kiourelli**[2], **Eftychia Evangelidou**[3], **Constantinos Papadopoulos**[4], **George Papadimas**[4], **Gerasimos Terzis**[1]

[1] *National and Kapodistrian University of Athens, Athens, Greece*
[2] *Harokopio University, Athens, Greece*
[3] *Konstantopouleio Geniko Nosokomeio Neas Ionias, Athens, Greece*
[4] *Eginition Hospital, Athens, Greece*

The present study aimed to investigate the effect of different eccentric-based power training volumes on body composition and lipidemic-glycemic profiles in females, as well as to explore the relationships between the change in glycemic-lipidemic profiles and the change in muscle fiber composition. Twenty-nine young females were assigned into three groups and performed 10 weeks of either 3 (LV), 6 (MV) or 9 (HV) sets/session of four 32 fast velocity eccentric-only half-squats against 70% of concentric 1RM, followed by 3 maximum countermovement jumps (CMJ) after each set. Body composition, vastus lateralis fiber type composition, and resting blood lipidemic and glycemic indices were evaluated 1 week before and after the training intervention.
Significant reductions in type IIx muscle fiber percentages of skeletal muscle's cross sectional area (%CSA) were found after moderate- and high-volume training, with concomitant increases in type IIa fibers. Power training induced beneficial changes in body composition and glycemic and lipidemic indices (glucose, insulin, HOMA-IR, triglycerides, total cholesterol, LDL-C, HDL-C and apoA1). Those changes are dependent on exercise volume with the higher training volumes showing the best improvement of the metabolic profile. Significant correlations were found between muscle fibers' percentage cross-sectional areas (%CSA) and resting glycemic lipidemic markers (r:-0.543–0.730, $p<0.05$). Higher Type I, IIA and lower IIX percentage cross-sectional areas are with healthier body composition and glycemic-lipidemic profiles. In addition, the training induced changes of glycemic-lipid markers were highly correlated with the changes of type IIa and IIx %CSA (r: -0.895 to 0.898, $p<0.05$). These results suggest that increased training volumes are needed for maximizing the potential benefits of power training programmes on body composition and glycemic-lipidemic profiles. Individuals experiencing the greatest increases in Type IIa and decreases in Type IIX muscle fibers cross-sectional areas after power training are those with the greatest beneficial changes in body composition, glycemic and lipidemic profiles.

P115

Pactamycin through the lens of context specificity for ribosome-acting antibiotics

Georgia G. Kournoutou[1], Elodie Leroy[2], Mélanie Gillard-Bocquet[2], George Dinos[1] & C. Axel Innis[2]

[1] Department of Biochemistry, School of Medicine, University of Patras, Patras, Greece

[2] Univ. Bordeaux, Centre National de la Recherche Scientifique, Institut National de la Santé et de la Recherche Médicale, ARNA, UMR 5320, U1212, Institut Européen de Chimie et Biologie, Pessac, France

Ribosome targeting antibiotics can stop bacterial cells from growing by interfering with its ability to synthesize proteins. Pactamycin, a known protein synthesis inhibitor, acts on the small ribosomal subunit, binding to the E site. Although pactamycin was originally viewed as a specific inhibitor of initiation, subsequent studies suggested that the drug interferes with translocation. However, this activity strongly depends on the nature of the pre-translocation complex, and specifically on the type of the A- site substrate.

To further explore the context specificity of pactamycin we performed whole cell proteomics using LC-MS/MS on *E.coli* cell extracts. Our analysis revealed a modification in the proteome of *E. coli* after treatment with the drug. In addition, we used a high-throughput procedure named inverse toeprinting, a recently developed method to identify peptide-encoding transcripts that induce ribosomal stalling in vitro. Inverse toe-printing experiments were performed using a completely random mRNA library of 15 codons (NNN_{15}). RNA exonuclease degrades the mRNA downstream of the leading ribosome making it possible to determine the position of the stalled ribosome on the mRNA with codon resolution. Inverse toeprinting experiments in the presence of pactamycin have shown preferential arrested modes of mRNA sequence, which varied among different mRNAs tested. These findings support the notion that pactamycin is a context specific inhibitor and more interestingly mRNA dependent, which is up to now the first case in the literature.

P116

Effect of *Hsp70* deficiency on psoriasis in mice

Maria Kostakou, Aikaterini Kalantidou, Maria Venihaki*
Laboratory of Clinical Chemistry, School of Medicine, University of Crete, Heraklion, Crete, Greece

Psoriasis is a chronic multifactorial disease which significantly affects 1-2% of the Caucasian population. It is characterized by changes in the physiology of the skin and accumulation of immune cells, $CD4^+$ and $CD8^+$ T-cells, dendritic cells, with a primary role on antigen presentation, and neutrophils. However, its pathogenesis remains unknown. Heat Shock proteins (HSPs) are expressed in most living cells and play a major role in immune responses as well as inflammation. Although there are few studies that try to clarify their role in the pathogenesis of skin diseases, in psoriasis the data are still unclear.

Purpose: Based on the above**,** the **purpose** of our study was to examine the importance of *Hsp70* deficiency on psoriasis, using the model of pharmaceutically-induced psoriasis in vivo.

Materials-Methods: Male wildtype mice (*Hsp70+/+)* and *Hsp70* deficient mice (*Hsp70-/-*) aged 8-12 weeks were used. Psoriasis was induced using imiquimod (IMQ) every day for 7 days and then the mice were sacrificed and blood and tissues were collected. The severity of psoriasis was assessed on the basis of PASI Score for mice.

Results: Macroscopically no differences were observed between the two genotypes according to PASI score. However, skin protein levels of IL-6 and TNF-α were elevated in *Hsp70-/-* mice compared to *Hsp70+/+ ones,* in contrast to IL-17A, where no significant difference was observed.

Conclusions: Our study shows that deletion of HSP70 gene does not alter the skin phenotype during Imiquimod-induced psoriasis. However, Hsp70 deficiency is associated with higher skin protein levels of proinflammatory cytokines (TNF-α and IL-6). Further, analyses of the mRNA levels of other cytokines as well as chemokines involved in psoriasis and the protein levels of HSP90 is in progress.

P117

Functional analysis of *HIF1A* knock-out cell lines reveals common and distinct HIF-1α roles under normoxia and hypoxia in hepatocellular carcinoma cells

Ioanna-Maria Gkotinakou[1], Christina Arseni[1], Martina Samiotaki[2], George Panayotou[2], George Simos[1], Ilias Mylonis[1]*

[1]*Laboratory of Biochemistry, Faculty of Medicine, University of Thessaly, Larissa, Greece*
[2]*Institute for Bio-innovation, BSRC "Alexander Fleming", Vari, 16672, Greece.*
**e-mail: mylonis@med.uth.gr*

Hepatocellular carcinoma (HCC) is one of the most prevalent cancers with limited therapeutic options and it is characterized by intra-tumoral oxygen deprivation (hypoxia), which promotes the activation of Hypoxia Inducible Factors (HIFs). HIF-1 orchestrates the transcriptional adaptation of cancer cells to hypoxia. Its oxygen-regulated HIF-1α subunit is often over-expressed in cancers and correlated with poor patient prognosis. To investigate the significance of HIF-1 for cancer cell adaptation to hypoxia, we used the CRISPR/Cas9 gene-editing method to generate both HeLa and Huh7 cell lines that do not express endogenous HIF-1α. Both types of *HIF1A*$^{-/-}$ cell lines were sensitive to oxygen deprivation. In contrast, when compared to wild-type Huh7 cells, *HIF1A*$^{-/-}$ Huh7 cells had increased death rate and reduced proliferation also under normoxia, suggesting that HIF-1α is essential for HCC cell survival irrespective of oxygen levels. Both wild-type and *HIF1A*$^{-/-}$ Huh7 cells grown under normoxia or hypoxia were then used in large scale comparative proteomic studies with LC-MS/MS. Our results indicate that in both normoxic and hypoxic *HIF1A*$^{-/-}$ Huh7 cells the expression of proteins involved in glycolysis, lipid biosynthesis and cell migration was reduced compared to wild-type Huh7 cells, in which most of the same proteins were upregulated under hypoxia. In addition, a different set of proteins, comprising components of mRNA processing, translation, and cell cycle regulation machineries, was downregulated only in hypoxic *HIF1A*$^{-/-}$ Huh7 cells compared to hypoxic wild-type Huh7 cells. Initial biochemical validation of our results has shown that lipid droplet accumulation, triglyceride production and cholesterol ester synthesis were reduced in both normoxic and hypoxic *HIF1A*$^{-/-}$ Huh7 cells compared to wild-type Huh7 cells. Our results suggest that HIF-1 plays a dual role in hepatocellular carcinoma cells: it mediates their metabolic phenotype under both normoxia and hypoxia, while it is also essential for sustaining cellular growth under hypoxia.

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P118

Effect of glucocorticoids, flavonoids, and non-steroidal anti-inflammatory drugs on TGF-β1-induced expression of fibrosis agents in human lung fibroblasts

K. Haikou[1], M-E. A. Christopoulou[1,2]*, A. J. Aletras[1]
[1]*Biochemistry, Biochemical Analysis & Matrix Pathobiology Res. Group, Laboratory of Biochemistry, Department of Chemistry, University of Patras, Greece;*
[2]*Department of Pneumology, Medical Center - University of Freiburg, Faculty of Medicine - University of Freiburg, Freiburg, Germany*

Lung diseases such as Idiopathic Pulmonary Fibrosis and Chronic Obstructive Pulmonary Disease are conditions that are characterized by inflammation and fibrosis. The cause is unknown, but these diseases can be distinguished from the destruction of normal tissue architecture. TGF-β1 has a central role in development of fibrosis and regulates IL-6 production by human fibroblasts. Standard treatment for said diseases are corticosteroids, like Dexamethasone, that prevent inflammation but there is uncertainty as to whether this treatment is effective. Quercetin is an herbal flavonoid and plays a role in anti-inflammation through negatively modulating pro-inflammatory factors, such as IL-6. In addition, it has been reviewed that non-steroidal anti-inflammatory drugs (NSAIDs) activate AMP-activated protein Kinase and this action might contribute to their cyclooxygenase (COX)-independent anti-inflammatory properties. The present study examines the effect of different treatments on the expression of TGF-β induced IL-6 production as well as the implication of AMPK activation. Lung fibroblasts derived for tissue of healthy human lung were isolated and cultured. Total RNA was isolated from the cells in order to study the expression of IL-6 by qPCR analysis, and in the culture medium the levels of IL-6 were determined with ELISA. All treatments caused significant suppression of TGF-β1-induced expression of IL-6. By using the AMPK inhibitor, Compound C, it was found that AMPK is not involved in the suppressive effect of all substances. TGF-β1-induced IL-6 expression is also potently suppressed by the free radical scavenger NAC, the src kinase inhibitor, PP2, and the EGFR inhibitor, AG1478. Prostaglandin PGE2 alone induced the expression of IL-6, which was suppressed by the presence of the src kinase inhibitor, PP2, and the EGFR inhibitor, AG1478. In conclusion, TGF-β1 appears to induce the expression of IL- 6 via a non-canonical signaling pathway and that the substances used affect said pathway.

P119

A sliding-elevator mechanism of transport underlies genetic modifications of substrate specificity in the NAT nucleobase-ascorbate transporters

Dimitris Dimakis, Yiannis Pyrris & George Diallinas
Department of Biology, National and Kapodistrian University of Athens, Panepistimioupolis 15784, Athens, Greece

Functionally characterized proteins of the NAT family function as plasma membrane nucleobase transporters in bacteria, plants, fungi and mammals[1]. UapA, a uric acid-xanthine/H^+ symporter of the model fungus ***Aspergillus nidulans***, which is the most studied NAT[2], has been crystallized as a dimer made of two protomers, each possessing 14 transmembrane segments (TMS) folded as two distinct parts, the ***core*** and ***dimerization*** domains[3]. The UapA crystal structure and a plethora of genetic, cellular and functional studies have supported that UapA operates via the so-called ***sliding-elevator mechanism***, where the movement of the motile core domain along the dimerization domain in each protomer mediates the influx substrates/H^+ to the cytosol[4]. Furthermore, homology modelling of the distinct conformations of UapA and Molecular Dynamics (MD), analysed under the light of functional and/or specificity mutations, suggested that a 'lock' mechanism imposed by electrostatic incompatibility of the 'empty' binding site in the core domain with the interface of the dimerization domain prevents uncontrolled sliding[5]. Through this mechanism, mutations that loosen the locking step lead to increased or less-controlled sliding, thus permitting the transport of weakly-binding ligands, other than physiological substrates, and consequently transform UapA into a more promiscuous transporter[5]. In the present work, we will present a novel systemic mutational analysis that shows that residues in TMS5 and TMS12 are critical for enlarging UapA specificity, further supporting the role of these segments in the sliding-elevator mechanism. In addition, we will show that modified UapA versions carrying specific mutations in the substrate binding site, rationally designed via evolutionary analysis of metazoan UapA homologues, exhibit altered function and substrate specificity. Overall, the present work paves the way for the modification of elevator-type transporters, which might prove important in the design of targeted pharmacological protocols based on elevator-type transporters.

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P120

A rapid and sensitive bioelectrical biosensor for the detection of the SARS-CoV-2 S1 spike protein based on membrane-engineered cells

Konstantina Karamanou, Georgios-Marios Papaioannou, Sophie Mavrikou*, Spyridon Kintzios
Laboratory of Cell Technology, Department of Biotechnology, Agricultural University of Athens, EU-CONEXUS European University, 11855 Athens, Greece

The current outbreak of the COVID-19 pandemic emerged the need for the development of biosensors that can identify SARS-CoV-2 timely and reliably. Thus, we developed a highly sensitive and selective Bioelectric Recognition assay-based biosensor able to detect the alterations in the electric potential of membrane-engineered fungal cells bearing the human spike S1 antibody after conjunction with the viral SARS-CoV-2 S1 protein. In the present study, we investigated the limits of detection of our proof-of-concept technology with different concentrations of SARS-CoV-2 S1 spike protein. Membrane-engineered *Debaryomyces* sp. cells have undergone transformation by double 2kV electroporation or by the addition of lysing enzymes from Trichoderma harzianum and afterwards 1kV electroporation. Suspension of *Debaryomyces* sp. cells was initially added at the top of the surface of gold screen-printed electrodes contained in a disposable sensor strip and then, SARS-CoV-2 Spike protein (S1) was added. The cells' response was immediately recorded as a time series of potentiometric measurements through a custom-made software. Our results revealed a concentration-dependent response during the assay after the application of increasing concentrations of the SARS-CoV-2 spike S1 protein to the membrane-engineered *Debaryomyces*/anti-S1 cells biosensor (10 ng/mL of human chimeric anti-S1 antibodies were used) with a linear pattern in the range 10 fg–1 μg/mL. This response was very rapid and clearly distinguishable from the response of non-electroporated *Debaryomyces* cells or electroporated but not enzyme-engineered cells. As a next step, we proceeded in confocal microscopy analysis, where the conjugation of SARS-CoV-2 spike S1 antibody was demonstrated in the cell surface. The results were considered promising, as we managed to detect the protein in very low S1 concentrations around 1fg/mL. These findings consider providing a promising tool for the efficient monitoring and the identification of asymptomatic cases as a new contribution to the global effort to manage the coronavirus pandemic.

P121

CDKN2A loss in circulating cell-free DNA as a non-invasive molecular marker for bladder cancer progression

Maria-Alexandra Papadimitriou[1], Katerina-Marina Pilala[1], Panagiotis Levis[2], Georgios Kotronopoulos[2], Konstantinos Stravodimos[2], Margaritis Avgeris[1,3,*], Andreas Scorilas[1]

[1] Department of Biochemistry and Molecular Biology, Faculty of Biology, National and Kapodistrian University of Athens, Athens, Greece

[2]First Department of Urology, "Laiko" General Hospital, School of Medicine, National and Kapodistrian University of Athens, 115 27 Athens, Greece

[3] Laboratory of Clinical Biochemistry - Molecular Diagnostics, Second Department of Pediatrics, School of Medicine, National and Kapodistrian University of Athens, "P. & A. Kyriakou" Children's Hospital, Athens, Greece

**e-mail: margaritis.avgeris@gmail.com*

Heterogeneity of bladder tumors and lack of personalized prognosis leads to bladder cancer (BlCa) patients' lifelong surveillance with invasive interventions, highlighting the need for modern molecular markers able to support personalized therapeutic decisions. Cell-free DNA (cfDNA) has emerged as a minimally invasive liquid biopsy tool, allowing early diagnosis, prognosis and treatment monitoring. Nowadays, research has unmasked a major contribution of copy number variations (CNVs) to tumorigenesis, with *CDKN2A* (Cyclin Dependent Kinase Inhibitor 2A) being one of the most frequently altered genes in BlCa. Herein, we have established a sensitive method for analyzing *CDKN2A* CNVs in serum cfDNA and we have evaluated *CDKN2A* cfDNA status in BlCa patients. Genomic DNA (gDNA) and cfDNA were extracted from 217 tumors and 190 serum samples of BlCa patients. *CDKN2A* status was evaluated by *in-house* developed qPCR assays using single-copy *LEPTIN* gene as endogenous reference control. Optimal cut-off for *CDKN2A* homozygous/hemizygous loss in gDNA was adopted by constructing a standard curve using T24 and RT112 cell lines with known *CDKN2A* deletions, spiked with known amount of diploid gDNA. Disease relapse and progression were assessed as clinical end-point events for survival analysis. Concerning bladder tumors, *CDKN2A* loss was significantly correlated with superior disease-free ($p=0.015$) and progression-free ($p=0.028$) survival of TaT1 (NMIBC) patients. Strikingly, the favorable prognostic utility of *CDKN2A* loss was maintained in serum cfDNA samples. In particular, *CDKN2A* cfDNA loss was positively associated with higher tumor stage ($p<0.001$) and grade ($p=0.001$), and significant higher risk for short-term progression of TaT1 patients (HR=2.658;$p=0.032$). Finally, multivariate models integrating *CDKN2A* cfDNA loss with disease clinical markers clearly offered superior risk-stratification and positive prediction of NMIBC progression to muscle-invasive disease. Our study identified *CDKN2A* loss as a powerful non-invasive independent predictor of superior outcome of TaT1 BlCa patients following treatment, supporting precision medicine decisions in patients management/monitoring.

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P122

Expression of the WD40-repeat Nucleoporins Nup43 and Seh1 in various systems for structural studies

Constantine Meliopoulos[1], Eftychia Filippopoulou[1], Antonis Athanasiadis[1], Benedeta Leka[1], Theodora Mantso[1], Penelope Mavromara[1], Katerina R. Katsani[1]*

[1]*Department of Molecular Biology & Genetics, Democritus University of Thrace, Alexandroupolis, Greece*

The Nuclear Pore Complexes (NPC) are taxonomically conserved supramolecular modular protein assemblies embedded in the eukaryotic nuclear envelope. NPCs have a cylindrical architecture with an eightfold rotational symmetry composed of multiple copies of about thirty proteins, named nucleoporins (Nups). Most nucleoporins are organized in discreet sub-complexes and share a small number of common folds[1].

The core structure of the NPCs, or core scaffold, is a cage-like structure composed of three coaxial rings (two outer rings and an inner ring)[1]. Biochemical and bioinformatic analyses have shown that scaffold nucleoporins share structural similarities with the vesicle coating complexes and intraflagellar transport system[2,3].

The well-characterized metazoan Nup107-Nup160 complex (Nup84 complex in fungi) or coat nucleoporin complex (CNC) forms the outer-ring scaffold of the NPCs. The coat nucleoporin complex has ten subunits assembled in a Y-shaped structure, therefore it is also referred to as the Y-complex. The short arm of the Y-complex consists of nucleoporin Nup85, and nucleoporins Seh1, and Nup43, which are WD40-repeat proteins that fold into β-propeller domain structures[1].

Nup43 and Seh1 remain understudied in both humans and model organisms. The *D. melanogaster* orthologues, DmNup43 and Nup44A/DmSeh1 exhibit high sequence, and, based on predictions, high structural similarity to their human counterparts. Even though the association Nup85-Seh1 is well characterized, the interaction between Nup43 and Seh1 is less addressed[4,5].

Seeking the expansion of knowledge to the *Drosophila* NPC and to intra-subcomplex interactions, we chose to express DmNup43 and DmSeh1 as His_6 proteins in prokaryotic *E. coli* and eukaryotic insect *Sf9* cells (using the Bac-to-Bac™ Baculovirus Expression System) for subsequent structural and interaction studies. We are currently expressing DmNup43 and DmSeh1 as individual subunits, and followingly, as a complex, using a dual expression vector. Next, we aim to express the additional coat nucleoporins Nup75/85, Nup37 and Nup160 as well.

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P123

The involvement of *gshA* and *grxB* genes of the glutaredoxin system of *Escherichia coli* in the transport and metabolism of maltose

Eirini-Angeliki Choremi[1], Martina Samiotaki[2], Alexios Vlamis-Gardikas[1*]
[1] *Department of Chemistry, University of Patras, Patras, 26504 Greece*
[2] *Institute of Bioinnovation, B.S.R.C. "Alexander Fleming", Vari, 16672 Greece*

In most living cells, redox homeostasis in the cytosol is maintained by the thiol-based thioredoxin and glutaredoxin (Grx) systems whose most known function is the reduction of ribonucleotide reductase. In the Grx system of *Escherichia coli* (*E. coli*), electrons are transferred from NADPH to glutathione reductase, glutathione (GSH), and the three/four Grxs. The most abundant and enzymatically active Grx is Grx2 (encoded by *grxB*), albeit with unknown function. All Grxs are reduced by GSH which is synthesized in a two-step reaction, the first catalyzed by glutamate-cysteine ligase (GshA protein, encoded by *gshA*). In the present work, we investigated the significance of the Grx system on the transport and metabolism of maltose in *E. coli* by comparing the proteomes of the *gshA* and *grxB* null mutants (*gshA*$^-$ and *grxB*$^-$ cells) and the wild type cells (MC1061 strain) after change of growth medium from glucose to maltose. Normally, maltose is imported to the periplasm by the outer membrane maltoporin where it binds to the maltose binding protein after which it is transferred to the cytosol by the maltose transporter $MalK_2FG$. Maltose is then broken down to glucose by MalP and MalQ. All *mal* genes are positively regulated by MalT. In the *grxB*$^-$ strain, that grew as well as the wild type, the expression of Cu^+ export systems, transporters and respiratory chain complex genes was upregulated, while there was no indication of any relation to maltose metabolism. MalK was upregulated in the *gshA*$^-$ strain whose growth on maltose lagged, suggesting a GSH-MalK dependent pathway for maltose metabolism, not related to Grx2. Other proteins upregulated in the *gshA*$^-$ strain were related to formaldehyde catabolism, amino acid biosynthesis and OxyR regulated genes reflecting the involvement of the Grx system in detoxification, general metabolism and oxidative stress respectively.

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Experimental studies of natural products as possible inhibitors of Aβ peptide aggregation in Alzheimer's disease

George D. Tzanetoulakos[1], Georgia I. Nasi[1], Panagiotis M. Spatharas[1], Virginia D. Dimaki[2], Yiorgos Paikopoulos[3,4], Niki Chondrogiani[3], Fotini N. Lamari[2], Vassiliki A. Iconomidou[1]*

[1]Section of Cell Biology and Biophysics, Department of Biology, School of Science, National and Kapodistrian University of Athens, Panepistimiopolis, Athens 157 01, Greece

[2]Laboratory of Pharmacognosy and Chemistry of Natural Products, Department of Pharmacy, University of Patras, 26504 Patras

[3]National Hellenic Research Foundation, Athens, Greece

[4]Department of Biochemistry and Biotechnology, University of Thessaly, Larissa, Greece

**e-mail: veconom@biol.uoa.gr*

Alzheimer's Disease (AD) is by far the most common cause of dementia worldwide and is characterized pathologically by the presence of two types of abnormal structures in the patients' brain, extracellular amyloid plaques and intracellular neurofibrillary tangles. The main component of the amyloid plaques is the Aβ peptide which derives from the proteolytic cleavage of the Amyloid Precursor Protein. For a considerable period of time, research in the field of AD was mainly focused on amyloid plaques, since it was believed that their formation leads to the development of the disease. However, recent studies have shown that the small oligomers of Aβ are its toxic and disease-relevant form. Therefore, inhibition of Aβ oligomerization is the new therapeutic approach for developing an effective treatment for AD. In recent years, natural products - including terpenoids, flavonoids, alkaloids and polyphenols - have been considered as potential inhibitors of Aβ aggregation, due to their antioxidant, anti-inflammatory and anti-amyloidogenic properties but also because of their ability to cross the Blood Brain Barrier (BBB). The aim of this study was to examine the ability of natural products to minimize or entirely block the aggregation of Aβ peptide *in vitro*.

Specifically, the effects of a kaurene diterpene extracted from native Greek plants of the genus Sideritis, on Aβ amyloid formation process, were examined using Molecular Biophysics methods, including Transmission Electron Microscopy, Congo Red birefringence and ThT fluorescence assays. Preliminary results of *in vivo* experiments confirm the *in vitro* results.

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Cross-talk between cell-matrix and cell-cell adhesions

Stavroula Arnokourou[1], Pinelopi A Nikolopoulou[1], Vassiliki Kostourou[1]*
[1]Biomedical Sciences Research Centre "Alexander Fleming", Institute of Bioinnovation, Athens, Greece

The vascular endothelium, a monolayer of endothelial cells (ECs), constitutes the inner cellular lining of arteries, veins and capillaries. It actively controls the degree of vascular relaxation and constriction, and the extravasation of solutes, fluid, macromolecules and hormones, as well as that of immune and blood cells (1). For all of this to happen the ECs form junctions with one another (adherent junctions) and adhere to the extracellular matrix at their basal site via another junction type (focal adhesions). Strict regulation of expression, distribution and function of ECs junctional proteins is pivotal for maintaining steady-state stability, integrity and barrier properties of vessel walls (2). This becomes more important if we take in consideration the fact that the two types of junctions are in constant crosstalk with one another. This means that when the structure or the function of one junction type is affected the other type gets affected too. Here we chose to study a key member of the focal adhesion proteins, talin. Talin is a large (270 kD) multidomain cytosolic protein that links integrins to F-actin via binding of its N-terminal FERM domain to integrin cytoplasmic domains and via two sites in its C-terminal flexible rod domain to F-actin (3). We present how the focal adhesions and adherent junctions are affected when the C-terminal F-actin binding domain of talin is missing. Furthermore we show how the incubation with different signaling molecules, that stabilize or destabilize these junctions, affects the ECs.

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P126

Effect of a Bone Morphogenetic Protein-2-derived peptide on MCF-7 cancer cell proliferation and on the expression of oncogene in osteoblasts and MCF-7 cells

Aglaia Mantsou*, Maria Pitou, Eleni Papachristou, Rigini M. Papi, Paraskevas Lamprou, Theodora Choli-Papadopoulou
Laboratory of Biochemistry, Department of Chemistry, Aristotle University of Thessaloniki, Greece
**e-mail: mantsouav@chem.auth.gr*

BMPs are the primary regulators of bone and cartilage formation and regeneration [1]. Zinc Finger Protein 217 (ZNF217) is a transcription factor and oncogene product that has been found to dysregulate Bone Morphogenetic Protein (BMP) signaling and induce invasion in breast cancer cells [2]. Also, it has been found that overexpression of *ZNF217* in a breast cancer cell line is associated with downregulation of BMP2 and upregulation of BMP4 and BMP receptors as well as induction of metastasis to the bone [3]. In this study, the effect of BMP-2 or an active peptide from its carboxyterminal region, AISMLYLDEN, on the expression of *ZNF217*, *BMP4* and CDK-inhibitor p21 gene, *CDKN1A,* was investigated in MCF-7 breast cancer cells. In addition, BMP-2 and the peptide were investigated in hDPSCs during osteogenic differentiation. MCF-7 cancer cells were treated with BMP-2 and different concentrations of the peptide AISMLYLDEN, all of which were effective against MCF-7 proliferation, as showed by BrdU assay. The peptide at a concentration of 22.6 ng/ml peptide was the most effective with regard to cell proliferation and gene expression, as *CDKN1A* and *BMP4* mRNA levels were substantially increased and *ZNF217* mRNA levels were decreased, 48 h after treatment. These results open ways for the concurrent use of the peptide AISMLYLDEN, as a novel therapeutic treatment, during conventional therapy in breast tumors with a metastatic tendency to the bones. The BMP-2 peptide, at 50 ng/mL, enhanced the osteogenic differentiation of hDPSCs more effectively than BMP-2, as evidenced but the mRNA levels of osteocalcin after 21 days, but it did not alter significantly the mRNA levels of *ZNF217*, *CDKN1A* and *BMP4*, which remained as expected of non-cancer cells.

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P127

miR-143/145 promoter methylation controls gene cluster's expression and prognostic value in urothelial bladder cancer

Katerina-Marina Pilala[1], Maria-Alexandra Papadimitriou[1], Konstantina Panoutsopoulou[1], Petros Barbarigos[1], Panagiotis Levis[2], Georgios Kotronopoulos[2], Konstantinos Stravodimos[2], Andreas Scorilas[1], Margaritis Avgeris[1,3,*]

[1] Department of Biochemistry and Molecular Biology, Faculty of Biology, National and Kapodistrian University of Athens, Greece

[2] First Department of Urology, "Laiko" General Hospital, School of Medicine, National and Kapodistrian University of Athens, Greece

[3] Laboratory of Clinical Biochemistry-Molecular Diagnostics, Second Department of Pediatrics, School of Medicine, National and Kapodistrian University of Athens, Greece

e-mail: margaritis.avgeris@gmail.com

Introduction: Bladder cancer (BlCa) represents the 2nd most common men urogenital cancer. Unfortunately, tumor heterogeneity and the absence of modern molecular diagnostics result to reduced prognostic efficacy and lack of personalized treatment decisions for the patients. Compelling evidence about deregulated levels of miR-143/145 cluster and their clinical significance to amelioration of patients' prognosis and risk stratification has already published by our group. Herein, we have studied the role of *MIR143/145* gene cluster methylation status on cluster's transcription and clinical value in BlCa.

Material and Methods: Total RNA and genomic DNA were extracted from 206 bladder tissue specimens. Genomic DNA underwent bisulfite conversion, followed by PCR amplification of specific *MIR143/145* promoter CpG sites. Methylation levels were quantified by pyrosequencing of the PCR products *via* PyroMark Q24. miR-143/145 levels were quantified by RT-qPCR, following 3'-terminal polyadenylation of total RNA. Progression-free survival (PFS) and overall survival (OS) were used to assess the survival outcome of NMIBC and MIBC patients, respectively.

Results: Bladder tumors displayed significantly increased methylation compared to normal adjacent tissues ($p<0.001$), along with an upward trend from distal to proximal promoter CpG sites. miR-143/145 levels are negatively correlated with promoter methylation, especially with TSS-20 (rs:-0.515,$p<0.001$) and TSS-29 (rs:-0.496,$p<0.001$) CpG sites upstream of *MIR145*. Regarding patients clinicopathological data, lower methylation levels were strongly correlated with muscle-invasive tumors ($p<0.001$), advanced tumor stages ($p=0.001$) and high-grade tumors ($p=0.005$) in accordance with miR-143/145 levels. Finally, survival analysis highlighted the significantly increased risk for disease progression (Kaplan-Meier: $p=0.017$; Cox:HR=2.991, $p=0.024$) and worse survival (Kaplan-Meier: $p=0.033$; Cox:HR=2.027, $p=0.039$) of TaT1 and MIBC patients overexpressing miR-143/145 due to reduced *MIR143/145* promoter methylation, respectively.

Conclusions: Promoter methylation of *MIR143/145* gene cluster controls miR-143/145 levels in bladder tumors, and constitutes a modern molecular marker to address clinical heterogeneity of disease prognosis and to support personalized treatment/monitoring decisions for BlCa patients.

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P128

The importance of long-range RNA-RNA interaction of the West Nile genome at the 5´UTR and 3´UTR

Maria Andreoglou*, Maria Bampali, Ioannis Karakasiliotis
Laboratory of Biology, Faculty of Medicine, Democritus University of Thrace, Alexandroupolis, Greece

Viral genome cyclization has been demonstrated to play a significant role in the replication of flaviviral RNA genome. Complementary sequences at the 5´ and 3´ UTRs of the viral RNA mediate long-range interactions that, along with local RNA-RNA interactions, generate the required conformation for RNA amplification. A significant number of the flavivirus RNA elements on both genome ends has been shown before to be essential for the genome cyclization. In this study, we investigated the presence of a new long-range interaction between the two ends of the West Nile Virus (WNV) genome that hasn't been reported before. Mutations at a 5´ UTR element of the WNV genome, that we have hypothesised to interact with its complementary sequence at the 3 ´ UTR, resulted in a significant decrease in the proliferation of the viral RNA. The analysis highlights the importance of the element for the efficient viral replication, while further analysis is required to assess the role of the mutated nucleotides in long-range end-to-end interaction for genome circularization. Compensatory nucleotide substitutions at the 3 ´ UTR are currently tested to confirm this interaction and its importance in viral replication.

P129

Identification of novel full-length cyclin-dependent kinase 4 (*CDK4*) transcript variants using a custom targeted nanopore sequencing approach

Konstantina Athanasopoulou, Panagiotis G. Adamopoulos, Panagiotis Tsiakanikas and Andreas Scorilas*
Department of Biochemistry and Molecular Biology, Faculty of Biology, National and Kapodistrian University of Athens, Greece

Cyclin-dependent kinases constitute a family of serine/threonine protein kinases that function as key regulators of cell cycle and signalling pathways. Notably, CDK4, a principal member of CDKs, forms complexes with cyclin D1 and regulates signalling cascades that enhance cellular proliferation, whereas deregulation of CDK4/D1 complex is implicated in multiple human malignancies. Besides the fundamental role of CDK4, the present study is seeking to explore the complete transcriptional landscape of *CDK4* in human malignancies, by identifying novel 5'- and 3'-untranslated regions (UTRs) as well as alternative splicing events between annotated exons that derive from *CDK4* pre-mRNA processing mechanisms. The identification of both 5'- and 3'-UTRs was accomplished by employing the direct RNA sequencing methodology, while the detection of 41 novel *CDK4* mRNA transcripts (*CDK4* v.2 – v.42) was carried-out using an *in-house* targeted nanopore sequencing approach. The relative expression levels of each novel *CDK4* transcript were assessed in an established panel of 51 human cancer cell lines, using custom qPCR-based assays with variant-specific primers. Furthermore, *in silico* analysis revealed a potential protein-coding capacity for 31 novel *CDK4* transcript variants, whereas the rest contain premature termination codons and hence represent non-coding RNAs, being candidates for nonsense-mediated mRNA decay (NMD) pathway. In conclusion, the current study presents a plethora of unidentified structural aspects of the human *CDK4* gene, by exploiting the state-of-the-art technology of nanopore sequencing. The detection and characterization of novel *CDK4* mRNA transcripts may further elucidate the exact functions and implications of CDK4 in cellular homeostasis and pathology.

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P130

Activation of the P2X7 receptor of red blood cells triggers MCP1 release. Modulatory effect of cholesterol depletion

Charalampos Papadopoulos[1,*], Ioannis Tentes, Konstantinos Anagnostopoulos
[1]*Laboratory of Biochemistry, Department of Medicine, Democritus University of Thrace, Alexandroupolis, Greece*
***Correspondence:** Charalampos Papadopoulos, Department of Medicine, Faculty of Health Sciences, Democritus University of Thrace, 68100 Alexandroupolis, Greece; E-mail: chapapa@med.duth.gr.

Background: P2X7 is a cation channel, activated by extracellular ATP. In human erythrocytes (red blood cells) P2X7 activation leads to cation flux and phosphatidylserine exposure. In rats activation of the P2X7 receptor of erythrocytes induces microvesicle release. Interestingly, erythrocyte microvesicles release chemokines. Thus, in this study we sought to investigate whether P2X7 activation of human erythrocytes results in chemokine release. Since the activity of P2X7 is largely regulated by membrane cholesterol, we also examined the effect of cholesterol depletion in the P2X7 activation-induced chemokine release.
Methods: Erythrocytes from whole blood were isolated from a healthy donor. Erythrocytes were incubated in RPMI 1640, supplemented with 10% FBS, 1% streptomycin/penicillin, for 30 minutes, at a concentration of 10^9 cells/ml, with or not the addition of methyl β-cyclodextrin at a concentration 5mM. The red blood cell-derived conditioned medium was discarded. Red blood cells were then incubated for 30 minutes, with or not the addition of BzATP at a concentration of 10μM. Finally, the red blood cell conditioned media were collected and the levels of the MCP1 were measured with ELISA.
Results: Activation of the P2X7 receptor augmented the release of the chemokine MCP1 (50.06 ± 1.04 pg / ml) compared to erythrocytes in the absence of stimulation (34.34 ± 4.4 pg/ml) ($p < 0.05$). Cholesterol depletion through methyl-β-cyclodextrin prior to stimulation, prevented in part the effect of P2X7 activation on MCP1 release from red blood cells (41.77 ± 1.00 pg/ml) ($p < 0.05$).
Conclusions: Our results indicate a novel pathway for red blood cells in systemic immunometabolism, where two metabolites (ATP and cholesterol) cooperate to induce an immune response.

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P131

Serglycin cooperates with cytokines to orchestrate LN-18 glioblastoma cells and fibroblasts behavior

Dimitra Manou[1], Zoi Karagiorgou[1], Panagiotis Fountas[1], Dimitris Kletsas[2], Aristidis Moustakas[3], Nikos K. Karamanos[1], Achilleas D. Theocharis[1]*

[1]*Biochemistry, Biochemical Analysis & Matrix Pathobiology Research Group, Laboratory of Biochemistry, Department of Chemistry, University of Patras, Greece*

[2]*Laboratory of Cell Proliferation & Ageing, Institute of Biosciences & Applications, NCSR "Demokritos", Athens, Greece*

[3]*Department of Medical Biochemistry and Microbiology, Science for Life Laboratory, Uppsala University, Sweden*

**e-mail: atheoch@upatras.gr*

The proteoglycan serglycin is an emerging modulator of tumor microenvironment involved in the regulation of both tumor and stromal cells, by controlling the bioavailability of important soluble factors, interacting with cell-surface receptors and recruiting various stromal cells. Glioblastoma multiforme (GBM) is the most aggressive type of brain tumor with high infiltrating capacity in the brain parenchyma. Serglycin has been found upregulated in GBM and is associated with low survival of GBM patients. Previous study of our laboratory indicated that serglycin is crucial for the overall aggressive phenotype of LN-18 GBM cells, as its suppression in LN-18 cells reduced proliferation and migration rates, invasion, tumorigenesis in vivo and colony formation ability, while simultaneously induce GBM to astrocytic differentiation. Additionally, LN-18 serglycin suppressed cells exhibit a lower proteolytic and inflammatory dynamic both in vitro & in vivo. Our recent data exhibit that the axis TGFβ1/TGFβRI is an important contributor of LN-18 aggressiveness only in the presence of serglycin, manipulating the stemness and inflammatory status of LN-18 cells. CNS fibroblasts are emerging players in CNS disease and inflammation. For that reason, we further investigated the effect of media derived from control LN-18 cells and serglycin-suppressed LN-18 cells on fibroblasts. Our data indicated that mainly the media from control LN-18 cells succeed to induce the proliferation and migration as well as the expression of numerous inflammatory mediators by activated fibroblasts. Blocking of TGFβ and IL-8 signaling in LN-18 cells and fibroblasts revealed only IL-8 signaling as crucial mediator of the cross-talk between LN-18 cells and fibroblasts. Our data depicts the participation of serglycin in tumorigenesis by affecting GBM cell signaling and cross-talk with stromal cells.

P132

Proteomic identification of the SLC25A46 interactome in transgenic mice expressing SLC25A46-FLAG

Vasiliki-Iris Perivolidi[1,2], Marina Mpoltsi[1], Vassiliki Kontou[1], Foteini Violitzi[1,2], Elisavet Ioannidou[2], Vagelis Rinotas[2], George Stamatakis[2], Martina Samiotaki[2], George Panayotou[2], Eleni Douni[1,2,*]

[1] *Laboratory of Genetics, Department of Biotechnology, Agricultural University of Athens, Iera Odos 75, 11855, Athens, Greece*

[2] *Institute for Bioinnovation, Biomedical Sciences Research Center "Alexander Fleming", Fleming 34, 16672, Vari, Greece*

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. 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 through interaction with core MICOS complex subunits MIC60 and MIC19 that are involved in cristae formation. In addition, SLC25A46 has been found to interact with mitochondrial fusion proteins OPA1, MFN1 and MFN2, suggesting a role for SLC25A46 also in mitochondrial dynamics. 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.

Our present work focuses on the identification of SLC25A46 interactome network in mouse tissues such as cerebellum, cerebrum, heart and thymus through immunoprecipitation with anti-FLAG beads and proteomic analysis with LC-MS/MS. Towards this scope, we generated and characterized transgenic mice expressing chimeric SLC25A46-FLAG protein. Our analysis identified 371 novel putative interactors of SLC25A46 and confirmed 17 known ones. The SLC25A46 interactome was compared among tissues, in order to reveal common and tissue-specific putative protein interactors for gaining insights about the pathophysiological role of SLC25A46 ***in vivo***. Co-immunoprecipitated proteins common in two or more tissues, mainly participate in mitochondrial activities such as oxidative phosphorylation (OXPHOS) and ATP production, active transport of molecules and metabolism. Tissue-specific co-immunoprecipitated proteins were enriched for synapse annotated proteins in cerebellum and cerebrum, for metabolic processes in heart, and for nuclear processes and proteasome in thymus.

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P133

An *in vivo* system for trans complementation of West Nile virus NS1 protein

Marios Dimitriou*, Katerina Kassela, Maria Bampali, Nikos Kesesidis, Maria Andreoglou, Stavroula Veletza, Ioannis Karakasiliotis
Laboratory of Biology, Department of Medicine, Democritus University of Thrace, Alexandroupolis, Greece

West Nile virus (WNV) is a neurotropic mosquito-borne virus, belonging to the genus ***Flavivirus***, family ***Flaviviridae***. Most human infections are asymptomatic or resemble a flu-like illness, but in less than 1% of cases, the infection leads to a potentially fatal neuroinvasive disease. The virus is considered a biosafety level 3 pathogen. To this date, there is no WNV vaccine that has been approved for use in humans. None of the proposed vaccine candidates has progressed to phase III clinical trials because of issues such as requirement of many boost immunizations, inability of establishing long-lasting immunity or failure of attenuating neuroinvasiveness and neurovirulence at the same time. WNV NS1 is a multifunctional protein that acts as a cofactor for viral replication and assembly inside the infected cell, while its secreted hexameric form plays a role in immune evasion. WNV lacking NS1 coding region is unable to replicate in cell culture, while NS1 can be provided ***in trans*** restoring replication. In this study, we have developed a cell culture system where a subgenomic WNV construct that lacks the coding region of WNV NS1 protein (WNV-ΔNS1 replicon) is efficiently replicating in Neuro2a cells stably expressing NS1. The effect was recapitulated using WNV-ΔNS1 pseudoparticles. In parallel, we have developed a mouse model expressing WNV NS1 protein under CD11b promoter in myeloid cells and is anticipated to trans-complement ***in vivo*** the WNV–ΔNS1 construct and eventually a ΔNS1 virus. The system may allow for ***in vivo*** experimentation under low biosafety and thus, it may boost the work on WNV vaccine worldwide. Furthermore, a system that may support the production and testing of a debilitated-attenuated virus may offer a platform for a safer WNV vaccine.

P134

Expression of HLA-class-I antigens and immune checkpoint molecules in prostate cancer cell lines before and after their engraftment in immunodeficient SCID mice

Theologos Sgouras[1*], Christos Kakouratos [1], Ioannis Lambrou[1], Erasmia Xanthopoulou[1], Alexandra Giatromanolaki [2], Michael I. Koukourakis

[1]*Department of Radiation Oncology, Democritus University of Thrace and University General Hospital of Alexandroupolis, Alexandroupolis, Greece*

[2]*Department of Pathology, Democritus University of Thrace and University General Hospital of Alexandroupolis, Alexandroupolis, Greece*

We investigated whether prostate cancer cells extracted from engrafted xenografts change their growth potential or have different levels of immune checkpoint inhibitory molecules, compared to the parental cells. More specifically, two commonly used prostate cancer cell lines, the hormone-resistant DU145 and the hormone-dependent 22Rv1, were cultured *in vitro*, and were subsequently subcutaneously injected to immunodeficient SCID mice, to achieve the formation of xenografts. When the tumor reached the size of 0.7x0.7 cm, the mice were euthanized, and primary cultures were formed, from the respective tumors. Then, the AlamarBlue® assay was applied to characterize the growth rate of the primary cultures, compared to the original ones. Western Blot analysis was also performed to determine whether the extracted primary cell lines, express altered levels of immune checkpoint inhibitory molecules compared to the original culture. We observed that the cell cultures obtained from xenografts show a significantly higher growth rate compared to the parental cultures. Furthermore, Western Blot analysis showed that the cells that were extracted from the xenografts, expressed higher levels of HLA-class-I antigens, PD-L1, CD80 and CD86, compared to the original cell lines. Further experiments are scheduled to identify the underlying mechanisms of the phenotypic changes induced by the cancer cell engraftment.

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P135

Biodegradation of environmental pollutants: A bioinformatics approach

Marina Giannakara, Vassiliki Lila Koumandou*
Department of Biotechnology, Agricultural University of Athens, Greece

Microbial biodegradation poses as a promising approach towards the removal of contaminants from the environment, due to the presence and abundance of bacteria in different and even extreme habitats[1]. Bioinformatics can contribute to this direction via the analysis of the available biological data in specialized databases[2]. Our study focuses on the C-P lyase and GOX enzymes which degrade glyphosate, a widely used pesticide. We collected the amino acid sequences from a broad range of microorganisms using the KEGG database and NCBI-BLAST, respectively. The conserved residues, identified via multiple alignment, were then mapped onto the 3-D structures of the enzymes, using PyMOL. Novel insights on the function of these enzymes will be discussed. We aim to investigate the conserved regions on their structure which are associated with their function, which will lead to the search for enzymes with an undiscovered potential to degrade glyphosate. This approach can yield insights into more enzymes important for bioremediation.

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P136

Development and application of a quantitative thin layer chromatography for sphingosine reveals unaltered sphingosine release from erythrocytes in Non-Alcoholic Fatty Liver Disease

Eleftheria Spourita[1], **Ioannis Tentes, Konstantinos Anagnostopoulos, Charalampos Papadopoulos***
[1]*Laboratory of Biochemistry, Department of Medicine, Democritus University of Thrace, Alexandroupolis, Greece*
Correspondence: Charalampos Papadopoulos, Department of Medicine, Faculty of Health Sciences, Democritus University of Thrace, 68100 Alexandroupolis, Greece; E-mail: chapapa@med.duth.gr.

Background: We recently developed a quantitative thin layer chromatography (TLC) for sphingomyelin and found that erythrocytes from NAFLD patients contain decreased sphingomyelin. This was inversely correlated to the release of MCP1, with unaffected sphingosine 1-phosphate release. To further elucidate the metabolism of sphingosine in erythrocytes, we developed and applied a quantitative TLC for sphingosine of erythrocyte-conditioned media (ECM) of NAFLD patients.
Methods: TLC analysis was done on a 10X10 cm chromatographic plate (TLC Silica gel 60 F254) using a mixture of chloroform/methanol/acetic acid/water (60/50/1/4) (v/v/v/v). For the determination of linearity and precision, limit of quantification (LOQ) and limit of detection (LOD), the analysis was repeated three times at concentrations of sphingosine standard: 5, 2.5, 1.25, μg/spot. The LOQ and LOD were expressed as 10*SD/slope and 3.3*SD/slope of the calibration curve, respectively. For the study of accuracy, we studied % recovery of 100%, 200% and 300% mass added in 2.5μg/spot. Specificity was calculated by comparing the retention factors of other lipids. The levels of sphingosine were quantified in spiked (5μg sphingosine) ECM ($5*10^7$ cells/ml) from 4 patients (3 men, 1 woman, aged 47.3±2 years) and 4 healthy controls (2 men, 2 women, aged 39,3±5,8 years). Lipids had been previously extracted with the Folch method.
Results: The linearity for the sum of the intensity of the Green+Blue colors is described by the equation y=-11.171x+353.25 (R^2=0,94). The interday precision is 0,21%, 1,65% and 0,44%. The accuracy is 97.0%±1.6%, 98.7%±8.4% and 94.5%±8.8%. The LOD and LOQ were 0.75μg and 1.21μg, respectively. No difference in the sphingosine levels was observed between the ECM from NAFLD patients (2.25±0.61 μg/ml) compared to the healthy controls (2.3±1 μg/ml).
Conclusion: Despite decreased sphingomyelin membrane content, sphingosine release from erythrocytes of NAFLD patients remains unaltered. These results indicate towards an accumulation of intermediate sphingolipids or augmented release of ceramide.

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Eisosome membrane domains are essential for the long-term survival of Quiescent yeasts

Amalia Megarioti[1], Tatiana Zakopoulou[1], Alexandros Athanasopoulos[1], Bruno André[2], Vicky Sophianopoulou[1] and Christos Gournas[1*]

[1]Microbial Molecular Genetics Laboratory, Institute of Biosciences and Applications, National Centre for Scientific Research "Demokritos", Patr. Grigoriou E & 27 Neapoleos St., 15341, Agia Paraskevi, Greece.
[2]Molecular Physiology of the Cell Laboratory, Université Libre de Bruxelles (ULB), IBMM, 6041 Gosselies, Belgium

Quiescence is the most common, but poorly-studied, cellular state of microorganisms in nature, essential for resistance and long-term survival under nutrient-limiting and stress conditions. The Plasma Membrane (PM) of yeast shows high degree of compartmentalization into several distinct domains, whose physiological role remains poorly understood. The Membrane Compartment of Can1 (MCC) or eisosome is the most studied domain and corresponds to furrow–like PM invaginations. We have previously shown that eisosomes expand, in number and size, at the beginning of the stationary phase, and at this stage they protect a sub-population of transporters from endocytosis, allowing efficient growth recovery after transient nutrient starvation. However, the physiological role of MCCs upon long-term starvation at the stationary phase and the entry in / survival during Quiescence remains unknown. In this work, we show that MCCs expand specifically in quiescent cells and are required for their long-term survival. More precisely, we provide evidence that MCCs expand, following glucose exhaustion, only in respiratory-active cells possessing cortical mitochondria. This MCC expansion requires not only glucose depletion by also active respiration and is deficient in *rho0* cells and mutants of the respiratory chain. Additionally, we show that MCC assembly in quiescent cells requires Lsp1, a protein previously considered inessential for MCC organization at the exponential phase. Most importantly, we provide evidence that strains lacking MCCs reach lower cell densities and show defective long-term survival of quiescent cells, phenotypes related to defective function of mitochondria. More specifically, in the absence of MCCs yeasts do not consume the ethanol produced by fermentation and display defective mitochondrial membrane potential. The molecular mechanisms provoking the above defects are under investigation. Our results uncover the dynamic changes in PM compartmentalization occurring during Quiescence and indicate the importance of this re-organization for respiration and long-term survival of quiescent yeasts.

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Immunomodulating activity of *Pleurotus eryngii* following its *in vitro* fermentation by human fecal microbiota

Marigoula Vlassopoulou[1,2], Ourania Konstanta[1,3], Evangelia N. Kerezoudi[2,4], Georgia Saxami[2], Evdokia K. Mitsou[2], Georgios Koutrotsios[5], Georgios I. Zervakis[5], Nikolaos Paschalidis[6], Panagiotis Georgiadis[1], Adamantini Kyriacou[2] and Vasiliki Pletsa[1]

[1] *National Hellenic Research Foundation, Institute of Chemical Biology, Athens, Greece*
[2] *Harokopio University, Department of Nutrition and Dietetics, Athens, Greece*
[3] *National & Kapodistrian University of Athens, School of Science, Department of Biology, Section of Botany, Greece*
[4] *Örebro University, School of Medical Sciences, Örebro, Sweden*
[5] *Agricultural University of Athens, Laboratory of General and Agricultural Microbiology, Athens, Greece*
[6] *Biomedical Research Foundation of the Academy of Athens (BRFAA), CyTOF Lab, Athens, Greece*

Edible mushrooms have been used for centuries in traditional medicine as enhancers of longevity and well-being. Current research has identified many of their health-promoting properties, ranging from antioxidant, antimicrobial and anticancer activities to immune enhancement and prebiotic action. These beneficial effects have been mainly attributed to mushroom polysaccharides, with beta-glucans being in the spotlight lately.
The immunomodulatory properties of *Pleurotus eryngii*, an edible mushroom rich in beta-glucans, selected due to its strong lactogenic effect and anti-genotoxic properties, were investigated. Lyophilized *P. eryngii* underwent *in vitro* static batch fermentation for 24 hours in the presence of human fecal microbiota from 5 healthy elderly volunteers (>60 years old). The fermentation supernatants (FSs) were administered (1% v/v) in U937-derived human macrophages for 6 and 24 hours. Gene expression of pro- and anti-inflammatory cytokines (IL-1β, IL-1Ra, IL-8, IL-10 and TNFα) was assessed via real-time PCR. The corresponding protein levels released in the cell culture supernatant were assessed via immunoassays. Additionally, the effect of FSs corresponding to selected volunteers on their PBMCs was investigated through Cytometry by time of flight (CyTOF) analysis.
The presence of *P.eryngii* in the fermentation process led to altered immune response, as indicated by the altered gene expression and protein levels of pro- (TNFα, IL1β, IL-8) and anti-inflammatory cytokines (IL10, IL1Rα) in the human macrophages. This finding was consistent for all volunteers. CyTOF analysis revealed that the products of the *in vitro* fermentation in the presence of *P.eryngii* affected the subpopulations distribution within monocytes in PBMCs.
Overall, the products of the *in vitro* fermentation of *P.eryngii* by human fecal inocula clearly affect the cytokine expression and alter the monocytes' distribution in PBMCs of healthy elderly volunteers, suggesting a potent immunomodulatory action for this edible mushroom. Further investigation is currently ongoing to correlate these findings with fermentation metabolites and fecal microbiota composition.

Keywords: *Pleurotus eryngii,* edible mushrooms, immunomodulation, cytokines

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Emerging SARS-CoV-2 protein drug targets: the multifaceted macro domain

Aikaterini C. Tsika[1], Nikolaos K. Fourkiotis[1] Angelo Gallo[1], Aikaterini I. Argyriou[1], Sridhar Sreeramulu[2], Harald Schwalbe[2] and Georgios A. Spyroulias[1]*

[1]*Department of Pharmacy, University of Patras, 26504 Patras, Greece*

[2]*Institute for Organic Chemistry and Chemical Biology, Johann Wolfgang Goethe-University Frankfurt, Max-von-Laue-Str. 7, 60438 Frankfurt, Germany*

ADP-ribosylation is a vital post-translational modification (PTM) associated with genome stability, oxidative stress, cell differentiation and proliferation and lately with immune response. In humans ADP-Ribosyl Transferases (ARTs) are responsible for ADP-ribose (ADPr) transfer onto substrates (proteins, nucleic acids, and small molecules) after NAD^+ conversion. Viral infections promote interferon (IFN) - stimulating gene expression (ISGs) during host cell defense. Amongst them, some genes of the poly-ADP-ribose-polymerases (PARPs), belonging to clade 1 of ARTs. Recently, has been found that viruses have evolved mechanisms to impede the cell immune responses based on ADP-ribosylation. Particularly, members of *Togaviridae* and *Coronaviridae* families contain a macro domain (MD) as a part of their multi-domain non-structural Protein 3 (nsP3). MDs form a structural family exhibiting a characteristic α/β/α sandwich fold and can be found in all kingdoms of life. They are divided in six classes depending on their functional characteristics. Viral MDs belong to Macro-D-type class having both free ADPr binding capacity as well as enzymatic activity to revert PARP-mediated ADP-ribosylation, proving their implication in the obstruction of the host defense mechanism. In humans there are at least 12 proteins containing 16 MDs, between them 3 PARPs having in total 7 MDs mainly members of macro-H2A like class. The discovery of inhibitors for viral MDs is a novel and promising approach for the limitations of viral infections. However, the possibility of affecting vital cellular paths due to binding to human macro domains could lead in various side effects. Herein we present a comparative structural and biochemical study of MDs from *Coronaviruses, Alphaviruses* and human. Our main goal is to detect putative distinct structural and functional characteristics that could be used in targeted compounds designing.

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Identification of Ataxin-10 as a novel regulator of HIF-2 transcriptional activity

Aikaterini Diseri[1], Ioanna Maria Gkotinakou[1], Christina Befani[1], Martina Samiotaki[2], George Panayotou[2] and Panagiotis Liakos[1]*
[1]Laboratory of Biochemistry, Faculty of Medicine, University of Thessaly, Biopolis 41500, Larissa, Greece
[2]Institute of Bioinnovation, BSRC "Alexander Fleming," Vari 16672, Greece
**email: pliakos@med.uth.gr*

Adaptive responses to hypoxic microenvironment are mediated by Hypoxia Inducible Factors (HIFs). The HIF family includes HIF-1 and HIF-2, both of which function as heterodimers, with an oxygen-regulated α subunit and a stably expressed β subunit, also known as ARNT. The expression of the second less-studied isoform (HIF-2α) is restricted to specific cell types and is involved in erythropoiesis, angiogenesis and metastasis. HIF-2**α** is controlled by oxygen-dependent as well as oxygen-independent mechanisms, such as phosphorylation. We have previously demonstrated that phosphorylation of HIF-2α by ERK1/2 under hypoxia stimulates the transcriptional activity of HIF-2 by inhibiting its CRM1-dependent nuclear export, revealing a novel regulatory mechanism of HIF-2 involving ERK1/2-dependent phosphorylation of HIF-2α. In this study, to further elucidate the HIF-2α regulation mechanism by ERK1/2 cascade, we investigated for new phosphorylation-dependent HIF-2α protein interactions.[1] Thus, we overexpressed and immunoprecipitated flag-tagged wild type HIF-2α or S672A HIF-2α mutant form that abolishes ERK1/2 mediated phosphorylation from HeLa cell extracts. We distinguished a protein that specifically bounds to S672A HIF-2α mutant form, which was identified by mass spectroscopy as Ataxin10. Ataxin10 is a protein that may function in neurogenesis, cell survival and differentiation.
To investigate the involvement of Ataxin 10 in the regulation of HIF-2α, its expression was suppressed in HeLa cells by small interfering RNA (siRNA)-mediated silencing. Treatment of cells with Ataxin10 siRNA under hypoxic conditions did not affect the expression levels of endogenous HIF-2α but reduced the mRNA expression levels of the HIF-2-specific target genes suggesting inhibition of HIF-2 transcriptional activity. These experiments were performed by RT-PCR and Luciferase assay. In corroboration, Flag-Ataxin10 overexpression experiments, led to an increase in HIF-2α transcriptional activity.
Our findings highlight Ataxin10 as a novel protein that regulates HIF2 activity and their association remains to be better investigated.

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Development of an efficient system for seamless integration of transgenes in mouse Embryonic Stem Cells

Konstantina-Maria Founta[1], Magdalini-Ioanna Tourkodimitri[1], Sylvia Bisti[1], Costis Papanayotou[1*]
[1]Biomedical Research Foundation Academy of Athens (Greece)

Expression of transgenes in recombinant cells is a common biotechnological tool with multiple practical uses. However, random integration of genes of interest (GOIs) is susceptible to unstable and variable expression and/or harmful alteration of the host genome. On the other hand, targeted insertion by homologous recombination is a time consuming and laborious. Using clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 and φC31 integrase mediated irreversible recombination, we have developed a two-transfection step method for seamless integration of a gene expression cassette into the Hipp11 “safe harbor” locus of mouse Embryonic Stem Cells (ESCs). This system can efficiently generate a fluorescent mESC line expressing high levels of tdTomato and can be potentially applied for the expression of a great variety of GOIs in different cell lines.
Mammalian cell expression systems are common biotechnological tools with multiple practical uses. However, random integration of genes of interest (GOIs) is susceptible to unstable and variable expression and/or harmful alteration of the host genome. On the other hand, targeted insertion of GOIs by homologous recombination is time consuming and laborious. Using clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 and φC31 integrase-mediated irreversible recombination, we have developed a two-transfection step method for seamless integration of a gene expression cassette into the Hipp11 “safe harbor” locus of mouse Embryonic Stem Cells (ESCs). Preliminary results suggest an efficient and accurate protocol for the generation of recombinant mESC lines, expressing high levels of fluorescent proteins. This system can potentially be applied for the expression of a great variety of GOIs in different cell lines.