WATCH ON LIVE STREAM

2021

8η Ημερίδα Νέων Επιστημόνων

Για 8η συνεχή χρονιά δίνεται η δυνατότητα σε νέους επιστήμονες να γνωριστούν, να παρουσιάσουν τα ερευνητικά τους αποτελέσματα και να ανταλλάξουν απόψεις και ιδέες για την έρευνα και το επαγγελματικό τους μέλλον.

25 ΝΟΕΜΒΡΙΟΥ **2021**

ΕΚΕΦΕ "ΔΗΜΟΚΡΙΤΟΣ"
ΠΑΤΡΙΑΡΧΟΥ ΓΡΗΓΟΡΙΟΥ & ΝΕΑΠΟΛΕΩΣ
ΑΓΙΑ ΠΑΡΑΣΚΕΥΗ, 153 10

210-65 03 572 (Πρατσίνης Χάρης)
210-36 34 944 (Era Ltd.)

hprats@bio.demokritos.gr
eebmbforum@gmail.com
info@era.gr

www.eebmbcongress.gr

Live Streaming
https://vimeo.com/event/1566689/embed
Poster Session:
https://zoom.us/j/94542825879

ΟΡΓΑΝΩΤΙΚΗ ΥΠΟΣΤΗΡΙΞΗ

ΥΠΟ ΤΗΝ ΑΙΓΙΔΑ: EEBMB

ΟΡΓΑΝΩΤΙΚΗ ΕΠΙΤΡΟΠΗ: ΓΙΑΡΔΟΓΛΟΥ ΠΑΝΑΓΙΩΤΑ - GUMENI SENTILJANA - ΘΑΝΟΥ ΕΙΡΗΝΗ - ΚΑΡΥΔΑ ΕΥΤΥΧΙΑ - ΚΟΛΟΚΟΤΡΩΝΗ ΑΝΘΗ - ΜΕΓΑΡΙΩΤΗ ΑΜΑΛΙΑ - ΜΟΥΣΤΑΚΑ ΚΑΛΛΙΟΠΗ - ΡΟΥΝΗ ΓΕΩΡΓΙΑ - ΣΤΙΒΑΡΟΥ ΘΕΟΔΩΡΑ
ΠΡΟΕΔΡΟΣ ΟΡΓΑΝΩΤΙΚΗΣ ΕΠΙΤΡΟΠΗΣ: ΠΡΑΤΣΙΝΗΣ ΧΑΡΗΣ

ABSTRACT BOOK

Short Talks

ST1

Differential DNA methylation in lymphoma subtypes

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; aivaliot@imbb.forth.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 development[1]. Lymphomas are a group of blood malignancies that develop from B, T/NK cells, featuring a wide range of heterogeneous subtypes[2]. 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 differential methylation in Diffused Large B-cell Lympoma's (DLBCL) subtypes vs. healthy controls, to identify genes with significant differential methylation.
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 platform[3]. 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 MSigDB[6]. 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 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. *Bibliography*

1. Skvortsova, K., Stirzaker, C. & Taberlay, P. The DNA methylation landscape in cancer. Essays in Biochemistry vol. 63 797–811 (2019).
2. Jaffe, E. S. Diagnosis and classification of lymphoma: Impact of technical advances. Semin. Hematol. ***56****, 30–36 (2019).*
3. Huber, W. et al. Orchestrating high-throughput genomic analysis with Bioconductor. Nat. Methods ***12****, 115–121 (2015).*
4. Ashburner, M. et al. Gene ontology: Tool for the unification of biology. Nature Genetics vol. 25 25–29 (2000).
5. Kanehisa, M. & Goto, S. KEGG: Kyoto Encyclopedia of Genes and Genomes. Nucleic Acids Research vol. 28 27–30 (2000).
6. Subramanian, A. et al. Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles. Proc. Natl. Acad. Sci. ***102****, 15545–15550 (2005).*

ST2

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.

ST3

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.

References:

1. *C. Dioli et al. "Tau-dependent suppression of adult neurogenesis in the stressed hippocampus". Mol Psychiatry. 2017 Aug;22(8):1110-1118. doi: 10.1038/mp.2017.103*
2. *C. Dioli et al. "Chronic stress triggers divergent dendritic alterations in immature neurons of the adult hippocampus, depending on their ultimate terminal fields". Transl Psychiatry. 2019 Apr 26;9(1):143. doi: 10.1038/s41398-019-0477-7*
3. *C. Dioli et al. "Adult neurogenic process in the subventricular zone-olfactory bulb system is regulated by Tau protein under prolonged stress" Cell Prolif 2021 Jul;54(7):e13027. doi: 10.1111/cpr.13027.*

ST4

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.

ST5

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.

ST6

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.

Acknowledgments: We acknowledge funding from the Operational Program "Human Resources Development, Education and Lifelong Learning" in the context of the project "Strengthening Human Resources Research Potential via Doctorate Research" (MIS-5000432), implemented by the State Scholarships Foundation (IKY).

ST7

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.

Acknowledgments:
This research is co-financed by Greece and the European Union (European Social Fund- ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning» in the context of the project "Reinforcement of Postdoctoral Researchers - 2nd Cycle" (MIS-5033021), implemented by the State Scholarships Foundation (IKY).

The work was supported also by the project "INSPIRED-The National Research Infrastructures on Integrated Structural Biology, Drug Screening Efforts and Drug target functional characterization" (MIS 5002550) which is implemented under the Action "Reinforcement of the Research and Innovation Infrastructure", funded by the Operational Programme "Competitiveness, Entrepreneurship and Innovation" (NSRF 2014-2020) and co-financed by Greece and the European Union (European Regional Development Fund).

ST8

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.

I. Tsika, A. C., Fourkiotis, N. K., Charalampous, P., Gallo, A., & Spyroulias, G. A. (2021). Biomolecular NMR assignments, 1–8. https://doi.org/10.1007/s12104-021-10052-5
II. Cantini, F., Banci, L., Altincekic, N., Bains, J. K., Dhamotharan, K., Fuks, C., Fürtig, B., Gande, S. L., Hargittay, B., Hengesbach, M., Hutchison, M. T., Korn, S. M., Kubatova, N., Kutz, F., Linhard, V., Löhr, F., Meiser, N., Pyper, D. J., Qureshi, N. S., Richter, C., … Spyroulias, G. A. (2020). Biomolecular NMR assignments, 14(2), 339–346. https://doi.org/10.1007/s12104-020-09973-4
III. Alhammad, Y., Kashipathy, M. M., Roy, A., Gagné, J. P., McDonald, P., Gao, P., Nonfoux, L., Battaile, K. P., Johnson, D. K., Holmstrom, E. D., Poirier, G. G., Lovell, S., & Fehr, A. R. (2021). Journal of virology, 95(3), e01969-20. https://doi.org/10.1128/JVI.01969-20

Acknowledgments:
This work is supported by the project "INSPIRED-The National Research Infrastructures on Integrated Structural Biology, Drug Screening Efforts and Drug target functional characterization" (MIS 5002550) which is implemented under the Action "Reinforcement of the Research and Innovation Infrastructure", funded by the Operational Programme "Competitiveness, Entrepreneurship and Innovation" (NSRF 2014-2020) and co-financed by Greece and the European Union (European Regional Development Fund).

ST9

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.

Funding: This work was supported by European Maritime and Fisheries Fund and Hellenic Ministry of Rural Development and Food in the frame of O.P. Maritime & Fisheries 2014-2020.

ST10

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.
References:
1) Dimou, S.; Georgiou, X.; Sarantidi, E.; Diallinas, G.; Anagnostopoulos, A.K. Profile of Membrane Cargo Trafficking Proteins and Transporters Expressed under N Source Derepressing Conditions in Aspergillus nidulans. J. Fungi 2021, 7, 560. https://doi.org/10.3390/ jof7070560

ST11

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.

BIBLIOGRAPHY:

1. Du, Fang, et al. "PINK1 Activation Attenuates Impaired Neuronal-like Differentiation and Synaptogenesis and Mitochondrial Dysfunction in Alzheimer's Disease Trans-Mitochondrial Cybrid Cells." *Journal of Alzheimer's Disease*, 2021, pp. 1–13., https://doi.org/10.3233/jad-210095.
2. Morton, Hallie, et al. "Defective Mitophagy and Synaptic Degeneration in Alzheimer's Disease: Focus on Aging, Mitochondria and Synapse." *Free Radical Biology and Medicine*, vol. 172, 2021, pp. 652–667., https://doi.org/10.1016/j.freeradbiomed.2021.07.013.
3. Van Giau, Vo, et al. "Mitochondrial Therapeutic Interventions in Alzheimer's Disease." Journal of the Neurological Sciences, vol. 395, 2018, pp. 62–70., https://doi.org/10.1016/j.jns.2018.09.033.
4. Zhao, Na, et al. "Physical Exercise May Exert Its Therapeutic Influence on Alzheimer's Disease through the Reversal of Mitochondrial Dysfunction via SIRT1–FOXO1/3–PINK1–Parkin-Mediated Mitophagy." Journal of Sport and Health Science, vol. 10, no. 1, 2021, pp. 1–3., https://doi.org/10.1016/j.jshs.2020.08.009.

ST12

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 analyze~~d 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.

Bibliography:
1) ~~Crisp et al~~., 2013. "Canine degenerative myelopathy: Biochemical characterization of superoxide dismutase 1 in the first naturally occurring non-human amyotrophic lateral sclerosis model". Experimental Neurology, 1-9, 248. 2) Story et al., 2020. "Canine Models of Inherited Musculoskeletal and Neurodegenerative Diseases". Frontiers in Veterinary Science, 1-21, 7.
3) Chandramouli and Qian, 2009. "Proteomics: Challenges, Techniques and Possibilities to Overcome Biological Sample Complexity". Human Genomics and Proteomics, 498-515, 48(6).
4) Alsemgeest et al., 1994. "Concentrations of serum amyloid-A (SAA) and haptoglobin (HP) as parameters of inflammatory diseases in cattle". The Veterinary quarterly, 21-23, 16(1).
5) Zweigner et al., 2006. "The role of lipopolysaccharide-binding protein in modulating the innate immune response". Microbes and Infection, 946-952, 8(3).
6) Canosa et al., 2019. "Correlation between Apolipoprotein E genotype and brain metabolism in amyotrophic lateral sclerosis". European Journal of Neurology, 306-312, 26(2).
7) Tanaka et al., 2013. "ITIH4 and Gpx3 are potential biomarkers for amyotrophic lateral sclerosis". Journal of Neurology, 1782-1797, 260(7).
8) Kjældgaard et al., 2018. "Amyotrophic lateral sclerosis: The complement and inflammatory hypothesis". Molecular Immunology, 14-25, 102.

ST13

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.

ST14

Optimization study of the biorecognition element of a membrane engineered cell-based bioelectric biosensor for the detection of the SARS-CoV-2 S1 spike protein: The case of *Debaryomyces sp*.

Konstantina Karamanou[1], Georgios-Marios Papaioannou[1], Sophie Mavrikou[1]*, Chris Tsoukas[2], Epaminondas Paplomatas[2], Spyridon Kintzios[1]

[1] *Laboratory of Cell Technology, Department of Biotechnology, Agricultural University of Athens, EU-CONEXUS European University, 11855 Athens, Greece*
[2] *Laboratory of Phytopathology, Department of Crop Science, 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. *Debaryomyce ssp.* cells were transformed with the spike S1 antibody. Cell suspensions were used as biorecognition elements at the top of the surface of gold screen-printed electrodes contained in a disposable sensor strip after application of several SARS-CoV-2 Spike protein (S1) concentrations. The cells' response was immediately recorded as a timeseries 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 transformed *Debaryomyces*/anti-S1 cells biosensor with a linear pattern in the range 10 fg–1 μg/mL.This response was very rapid and clearly distinguishable from the response of control cells.The biosensor 's responses were considered statistically significant in comparison with the control, as we managed to obtain responses fromS1 protein concentrations down to 1fg/mL. These findings are considered to provide 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.

ST15

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*. \

Posters

FP1

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.

FP2

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, D*emocritus 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.

FP3

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.

This research is financed by Hellenic Foundation for Research & Innovation (HFRI 405-Public grant).

FP4

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.

This work was supported by the project "TRANSITION: TRANSlating the complexIty of melanoma diagnosis into raTional therapeutic stratificatiON" (MIS 5031295) which is implemented under the Action "Reinforcement of the Research and Innovation Infrastructure", funded by the Operational Programme "Competitiveness, Entrepreneurship and Innovation" (NSRF 2014-2020) and co-financed by Greece and the European Union (European Regional Development Fund).
G. Kontogianni's research is co-financed by Greece and the European Union (European Social Fund- ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning» in the context of the project "Reinforcement of Postdoctoral Researchers - 2nd Cycle" (MIS-5033021), implemented by the State Scholarships Foundation (IKY).

FP5

STUDY ON PHOTOSYNTHESIS OF RECOMBINANT CYANOBACTERIA LACKING PHYCOCYANIN

Giakoumidaki-Voyiatzi Aikaterini[1*], Amanatidou Elisavet[2], Melis Anastasios[3], Stamatakis Kostas[1]
[1]Biophysics and Biotechnology of Membranes Laboratory, Institute of Biosciences & Applications, N.C.S.R. "Demokritos", Patr. Grigoriou & Neapoleos 27, 153 41, Ag. Paraskevi, Athens
[2]Department of Chemical Engineering, University of Western Macedonia, Koila, Kozani 50100, 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 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(S6803) 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.

[1] Chaves JE, Rueda-Romero P, Kirst H, Melis A (2017). ACS Synth. Biol. 12:2281–2292
[2] Chaves JE, Melis A (2018) Appl Microbiol Biotechnol (2018). 102:6451–6458

Acknowledgments: Partnership and Cooperation Agreement (NSRF) 2014-2020, European Regional Development Fund (ERDF) Development of New Innovative Energy Technologies of Low Carbon Footprint for the Enhancement of Excellence in the Region of Western Macedonia - SUB-PROJECT 01_uowm,. MIS code 5047197 of The Operational Program "Competitiveness, Entrepreneurship & Innovation" (EPAnEK) co-financed by Greece and the European Union

FP6

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.

Acknowledgments: Partnership and Cooperation Agreement (NSRF) 2014-2020, European Regional Development Fund (ERDF) Development of New Innovative Energy Technologies of Low Carbon Footprint for the Enhancement of Excellence in the Region of Western Macedonia - SUB-PROJECT 01_uowm,. MIS code 5047197 of The Operational Program "Competitiveness, Entrepreneurship & Innovation" (EPAnEK) co-financed by Greece and the European Union.

FP7

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. Colocalization 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.

FP8

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.

Acknowledgments: We acknowledge funding from the Operational Program "Human Resources Development, Education and Lifelong Learning" in the context of the project "Strengthening Human Resources Research Potential via Doctorate Research" (MIS-5000432), implemented by the State Scholarships Foundation (IKY).

FP9

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.

Acknowledgements

This research was partly supported by the EU Horizon 2020 project RISE-2014, action No. 645756 "GLYCANC-Matrix glycans as multifunctional pathogenesis factors and therapeutic targets in cancer", as well as, by the projects "A Greek Research Infrastructure for Visualizing and Monitoring Fundamental Biological Processes (BioImaging-GR)" (MIS 5002755) and "Target Identification and Development of Novel Approaches for Health and Environmental Applications

FP10

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.

Acknowledgments
This research has been co-financed by the European Union and Greek national funds through the Operational Program "Competitiveness, Entrepreneurship and Innovation", under the call "RESEARCH – CREATE – INNOVATE" (project code: T1EDK-00353)

FP11

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.

References

1.Pandey et al. (2017) *Front Plant Sci* 8: 537
2.Castro-Moretti et al. (2020) *Metabolites* 10:52
3.Reddy et al. (2009) *Adv Virus Res 75:185-220*
4.Allen et al. (2017) *Plant Heal Prog 18:19-27*

Acknowledgements
This work was supported by the project PlantUp of Operational Programme Competitiveness, Entrepreneurship, and Innovation 2014-2020 (EPAnEK).

FP12

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.

References

[1] *N. Vassilaki et al., "Role of the Hepatitis C Virus Core+1 Open Reading Frame and Core cis-Acting RNA Elements in Viral RNA Translation and Replication," J. Virol., vol. 82, no. 23, pp. 11503–11515, Dec. 2008, doi: 10.1128/JVI.01640-08.*

[2] *S. Moustafa, I. Karakasiliotis, and P. Mavromara, "Hepatitis C Virus core+1/ARF Protein Modulates the Cyclin D1/pRb Pathway and Promotes Carcinogenesis," J. Virol., vol. 92, no. 9, May 2018, doi: 10.1128/JVI.02036-17.* [3] High levels of HCV core+1 antibodies in HCV patients with hepatocellular carcinoma.

Dalagiorgou G, Vassilaki N, Foka P, Boumlic A, Kakkanas A, Kochlios E, Khalili S, Aslanoglou E, Veletza S, Orfanoudakis G, Vassilopoulos D, Hadziyannis SJ, Koskinas J, Mavromara P.J Gen Virol. 2011 Jun;92(Pt 6):1343-1351. doi: 10.1099/vir.0.023010-0.

FP13

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.

Bibliography

1: Kampinga, Harm H., et al. "Guidelines for the nomenclature of the human heat shock proteins." Cell Stress and Chaperones 14.1 (2009): 105-111.

2: Richter, Klaus, and Johannes Buchner. "Hsp90: chaperoning signal transduction." Journal of cellular physiology 188.3 (2001): 281-290.

3: Trepel, Jane, et al. "Targeting the dynamic HSP90 complex in cancer." Nature reviews cancer 10.8 (2010): 537-549. 4: Brown, Mark A., et al. "Hsp90—from signal transduction to cell transformation." Biochemical and biophysical research communications 363.2 (2007): 241-246.

5: Ciocca, Daniel R., and Stuart K. Calderwood. "Heat shock proteins in cancer: diagnostic, prognostic, predictive, and treatment implications." Cell stress & chaperones 10.2 (2005): 86.

6: Whitesell, Luke, and Susan L. Lindquist. "HSP90 and the chaperoning of cancer." Nature Reviews Cancer 5.10 (2005): 761-772.

7: Jego, Gaëtan, et al. "Targeting heat shock proteins in cancer." Cancer letters 332.2 (2013): 275-285. 8: Georgakis, Georgios V., Yang Li, and Anas Younes. "The heat shock protein 90 inhibitor 17-AAG induces cell cycle arrest and apoptosis in mantle cell lymphoma cell lines by depleting cyclin D1, Akt, Bid and activating caspase 9." British journal of haematology 135.1 (2006): 68-71.

9: Georgakis, Georgios V., et al. "Inhibition of Heat Shock Protein 90 Function by 17-Allylamino-17-Demethoxy-Geldanamycin in Hodgkin's Lymphoma Cells Down-Regulates Akt Kinase, Dephosphorylates Extracellular Signal–Regulated Kinase, and Induces Cell Cycle Arrest and Cell Death." Clinical cancer research 12.2 (2006): 584-590.

10: Tabe, Yoko, et al. "MDM2 antagonist nutlin-3 displays antiproliferative and proapoptotic activity in mantle cell lymphoma." Clinical Cancer Research 15.3 (2009): 933-942.

11: Psatha, Konstantina, et al. "Differential proteomic analysis of the reactivated p53 via Nutlin-3a, in 3 different types of human lymphomas." (2016).

FP14

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.

***Funding**: This research has been co-financed by Greece and the European Union through the Operational Program "Competitiveness, Entrepreneurship and Innovation" (NSRF 2014–2020), under the Action "RESEARCH - CREATE – INNOVATE", in the context of the project "New therapies aiming to improve the atheroprotective and immunomodulatory properties of high-density lipoprotein (HDL) for the treatment of autoimmune and cardiovascular diseases" (project code T2EDK-02361, MIS 5067590).*

FP15

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.

References

1. Guo S, Qian W, Cai J, Zhang L, Wery JP, Li QX. Molecular Pathology of Patient Tumors, Patient-Derived Xenografts, and Cancer Cell Lines. Cancer Res. 2016;76(16):4619-4626. doi:10.1158/0008-5472.CAN-15-3245 Brady, L., Gil da Costa, R. M., Coleman, I. M., Matson, C. K., Risk, M. C., Coleman, R. T., & Nelson, P. S. (2020). A comparison of prostate cancer cell transcriptomes in 2D monoculture vs 3D xenografts identify consistent gene expression alterations associated with tumor microenvironments. The Prostate, 80(6), 491-499.

Vincent, K. M., Findlay, S. D., & Postovit, L. M. (2015). Assessing breast cancer cell lines as tumour models by comparison of mRNA expression profiles. Breast Cancer Research, 17(1), 114.

Zachari MA, Chondrou PS, Pouliliou SE, Mitrakas AG, Abatzoglou I, Zois CE, Koukourakis MI. Evaluation of the alamarblue assay for adherent cell irradiation experiments. Dose Response. 2013 Sep 12;12(2):246-58. doi: 10.2203/dose-response.13-024.Koukourakis. PMID: 24910583; PMCID: PMC4036397.

Zaidi MR, Merlino G. The two faces of interferon-γ in cancer. Clin Cancer Res. 2011 Oct 1;17(19):6118-24. doi:

10.1158/1078-0432.CCR-11-0482. Epub 2011 Jun 24. PMID: 21705455; PMCID: PMC3186825. Kuwata T, Yanagihara K, Iino Y, Komatsu T, Ochiai A, Sekine S, Taniguchi H, Katai H, Kinoshita T, Ohtsu A. Establishment of Novel Gastric Cancer Patient-Derived Xenografts and Cell Lines: Pathological Comparison between Primary Tumor, Patient-Derived, and Cell-Line Derived Xenografts. Cells. 2019 Jun 14;8(6):585. doi: 10.3390/cells8060585. PMID: 31207870; PMCID: PMC6627523.
Sugiura D, Maruhashi T, Okazaki IM, Shimizu K, Maeda TK, Takemoto T, Okazaki T. Restriction of PD-1 function by cis-PD-L1/CD80 interactions is required for optimal T cell responses. Science. 2019 May 10;364(6440):558-566. doi: 10.1126/science.aav7062. Epub 2019 Apr 18. PMID: 31000591.

FP16

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

Panagiota ~~G~~iardoglou[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.

This research is co-financed by Greece and the European Union (European Social Fund- ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning» in the context of the project "Strengthening Human Resources Research Potential via Doctorate Research" (MIS-5000432), implemented by the State Scholarships Foundation (IKY)

FP17

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.

FP18

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.

Bibliography

Validation of a protocol for the extraction and quantitative analysis of sphingomyelin in erythrocytes of patients with Non-Alcoholic Fatty Liver disease, C. Papadopoulos, et al., accepted by Journal of Planar Chromatography-Modern TLC, available on https://doi.org/10.1101/2021.04.16.440128

Red blood cell-conditioned media from patients with non-alcoholic fatty liver disease contain increased MCP1 and induce TNF-a release. C. Papadopoulos et al., accepted by Reports of Biochemistry and Molecular Biology, available on https://doi.org/10.1101/2021.05.10.21256939

FP19

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.

FP20

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.

FP21

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 ($LN\text{-}18^{shSRGN}$) characterized by reduced proliferation and migration rates, as well as tumorigenesis *in vivo*. $LN\text{-}18^{shSRGN}$ 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.

FP22

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

FP23

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], ~~Dimi~~tris 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

FP24

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.

I. Rack JG, Perina D, Ahel I. Annu Rev Biochem. 2016 Jun 2;85:431-54.

II. Lüscher B, Bütepage M, Eckei L, Krieg S, Verheugd P, Shilton BH. Chem Rev. 2018 Feb 14;118(3):1092-1136. III. Tsika AC, Melekis E, Tsatsouli SA, Papageorgiou N, Maté MJ, Canard B, Coutard B, Bentrop D, Spyroulias GA.. J Mol Biol. 2019 May 31;431(12):2283-2297.

IV. Cantini F, Banci L, Altincekic N, Bains JK, Dhamotharan K, Fuks C, Fürtig B, Gande SL, Hargittay B, Hengesbach M, Hutchison MT, Korn SM, Kubatova N, Kutz F, Linhard V, Löhr F, Meiser N, Pyper DJ, Qureshi NS, Richter C, Saxena K, Schlundt A, Schwalbe H, Sreeramulu S, Tants JN, Wacker A, Weigand JE, Wöhnert J, Tsika AC, Fourkiotis NK, Spyroulias GA. Biomol NMR Assign. 2020 Oct;14(2):339-346.

V. Rack JGM, Zorzini V, Zhu Z, Schuller M, Ahel D, Ahel I. Open Biol. 2020 Nov;10(11):200237.

Acknowledgments: This work is supported by the project "INSPIRED-The National Research Infrastructures on Integrated Structural Biology, Drug Screening Efforts and Drug target functional characterization" (MIS 5002550) which is implemented under the Action "Reinforcement of the Research and Innovation Infrastructure", funded by the Operational Programme "Competitiveness, Entrepreneurship and Innovation" (NSRF 2014-2020) and co-financed by Greece and the European Union (European Regional Development Fund).

FP25

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.

FP26

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.

FP27

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], ~~Leo~~nid~~as 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.

1. Ruiz-Ortega, M., et al., Targeting the progression of chronic kidney disease. *Nat Rev Nephrol,* **2020**. *16*(5): p. 269-288. doi:10.1038/s41581-019-0248-y

FP28

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.

Acknowledgements:
ΠΡΑΞΗ/ΔΡΑΣΗ/ΕΡΓΟ: Ενιαία Δράση Κρατικών Ενισχύσεων Έρευνας, Τεχνολογικής Ανάπτυξης & Καινοτομίας «ΕΡΕΥΝΩ – ΔΗΜΙΟΥΡΓΩ –ΚΑΙΝΟΤΟΜΩ Β ΚΥΚΛΟΣ» του Ε.Π.«Ανταγωνιστικότητα, Επιχειρηματικότητα και Καινοτομία (ΕΠΑνΕΚ)», ΕΣΠΑ 2014 – 2020.

FP29

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.

FP30

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.

FP31

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

[1]K. Haikou, [1,2]M-E. A. Christopoulou*, [1]A. J. Aletras

[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.

FP32

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.

Acknowledgments
Hellenic Foundation for Research & Innovation (H.F.R.I.) for 2nd Scholarship for PhD Candidates

FP33

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.

The research 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-cost research equipment grant" (Project Number: HFRI-FM17-1611).

FP34

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.

Funding: EU and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH –CREATE –INNOVATE (project code: MIS 5031816)

FP35

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] 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.

References

*1. Yang, K. et al. ER exit sites in Drosophila display abundant ER-Golgi vesicles and pearled tubes but no megacarriers. Cell Rep. **36**, (2021).*

*2. G, Z. et al. The structure of the COPII transport-vesicle coat assembled on membranes. Elife **2**, (2013). 3. Gomez-Navarro, N. & Miller, E. Protein sorting at the ER–Golgi interface. J. Cell Biol. **215**, 769–778 (2016). 4. Thomas, L. L. & Fromme, J. C. Extensive GTPase crosstalk regulates Golgi trafficking and maturation. Curr. Opin. Cell Biol. **65**, 1–7 (2020).*

5. Glick, B. S. & Nakano, A. Membrane Traffic Within the Golgi Apparatus. Annu. Rev. Cell Dev. Biol. ***25****, 113 (2009). 6. HY, G., J, K. & MG, L. Unconventional secretion of transmembrane proteins. Semin. Cell Dev. Biol.* ***83****, 59–66 (2018).*
7. Dimou, S. et al. Translocation of nutrient transporters to cell membrane via Golgi bypass in Aspergillus nidulans. EMBO Rep. ***21****, (2020).*
8. Chatterjee, S., Choi, A. J. & Frankel, G. A systematic review of Sec24 cargo interactome. Traffic (2021) doi:10.1111/TRA.12817.

FP36

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.

FP37

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.

1.Chachami, et al, Mol.Cell.Prot. 2019, 18, 1197–1209.

The research 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: 1460 to G.C.).

FP38

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 (0.1μM, 1μM and 10μM) 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.

Acknowledgement
This work was supported by the project "Bioimaging-GR" (MIS5002755) implemented under the Action "Reinforcement of the Research and Innovation Infrastructure", funded by the Operational Programme "Competitiveness, Entrepreneurship and Innovation" (NSRF 2014–2020) and co-financed by Greece and the EU (European Regional Development Fund).

References
1. Lakshmi V. et al., Reports in Organic Chemistry 2016:6 1-24
2. Prousis K.C. et al., Dyes and Pigments 2020, 175, 108047

FP39

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.

Acknowledgements
The research work was supported by the Hellenic Foundation for Research and Innovation (HFRI) and the General Secretariat for Research and Technology (GSRT).

FP40

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.

FP41

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.

ΧΟΡΗΓΟΙ 8ΗΣ ΗΜΕΡΙΔΑΣ ΝΕΩΝ ΕΠΙΣΤΗΜΟΝΩΝ

Lab Supplies
Scientific

LIFE SCIENCE CHEMILAB
LSC

analab

metrolab
DELIVERING VALUE

ΔΗΜΟΚΡΙΤΟΣ

ΤΖΑΡΤΟΣ
ΝΕΥΡΟΔΙΑΓΝΩΣΤΙΚΗ

ELPEN

Leica
MICROSYSTEMS
ΑΝΑΛΥΤΙΚΕΣ ΣΥΣΚΕΥΕΣ Α.Ε.
Δρ Κ.Ι. ΒΑΜΒΑΚΑΣ - ΕΠΙΣΤΗΜΟΝΙΚΟΣ ΕΞΟΠΛΙΣΜΟΣ

ΠΝΟΗ ΕΡΓΑΣΤΗΡΙΟΥ Α.Ε.
EST. 2004
Pnoi lab