9η Ημερίδα ΝΕΩΝ ΕΠΙΣΤΗΜΟΝΩΝ ΕΕΒΜΒ

Νέοι Επιστήμονες
Ελληνική Εταιρεία Βιοχημείας και Μοριακής Βιολογίας (ΕΕΒΜΒ)

01.12.2022

ΠΑΤΡΑ

Συνεδριακό &
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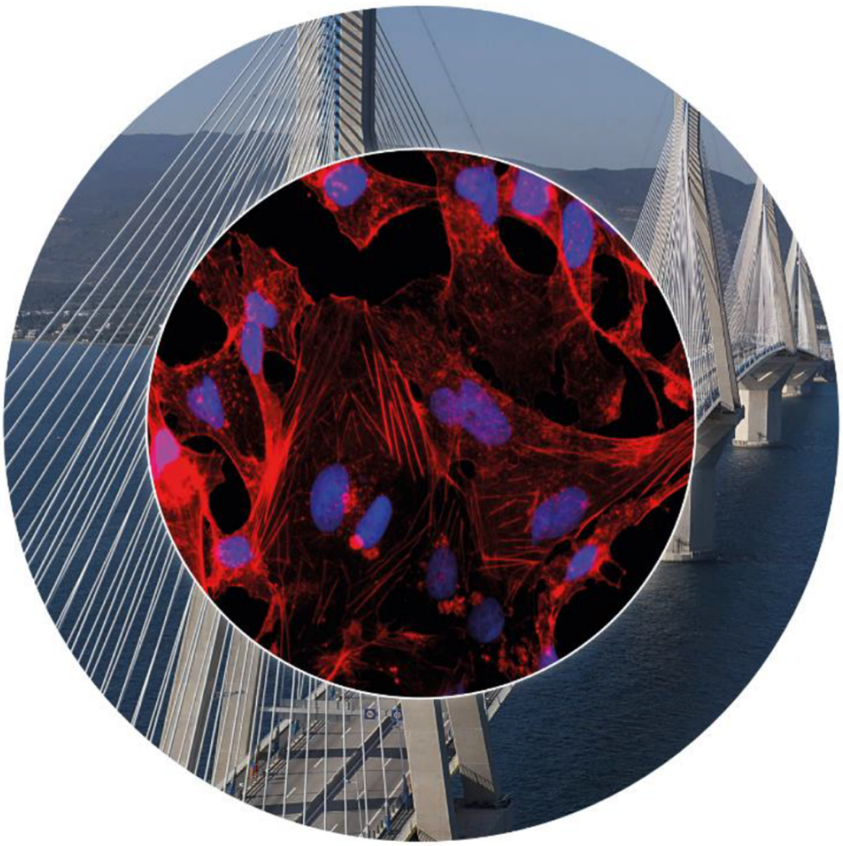

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Πρόσκληση

Με ιδιαίτερη χαρά σας ανακοινώνουμε τη διεξαγωγή της 9ης Πανελλήνιας Ημερίδας Νέων Επιστημόνων της Ελληνικής Εταιρείας Βιοχημείας και Μοριακής Βιολογίας (ΕΕΒΜΒ) που θα πραγματοποιηθεί στο Συνεδριακό & Πολιτιστικό Κέντρο του Πανεπιστημίου Πατρών, την Πέμπτη 1 Δεκεμβρίου 2022.

Η Ημερίδα Νέων Επιστημόνων αποτελεί πλέον έναν πολύ επιτυχημένο θεσμό που διεξάγεται για 9η συνεχή χρονιά παράλληλα με το 72ο Συνέδριο της ΕΕΒΜΒ και οργανώνεται ανεξάρτητα από μία επιτροπή νέων ερευνητών που δραστηριοποιούνται σε Πανεπιστήμια και Ινστιτούτα της χώρας.

Βασικό στόχο της Ημερίδας αποτελεί η ανάδειξη των ερευνητικών προσπαθειών νέων ερευνητών της Ελλάδας και του εξωτερικού που δραστηριοποιούνται στον τομέα των Βιοεπιστημών, μέσα από προφορικές και αναρτημένες εργασίες. Η Ημερίδα θα αποτελέσει το χώρο για αλληλεπίδραση με άλλους νέους ερευνητές, αλλά και με τους προσκεκλημένους ομιλητές, μέσα από συζητήσεις και στρογγυλές τράπεζες, δημιουργώντας νέες προοπτικές συνεργασίας και ανοίγοντας νέους ορίζοντες στην έρευνα.

Προσβλέπουμε στη δυναμική συμμετοχή σας.

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Πρόεδρος Οργανωτικής Επιτροπής

Οργανωτική Επιτροπή

Πρόεδρος

Ζωή Πιπερίγκου, Τμήμα Χημείας, Πανεπιστήμιο Πατρών

Μέλη

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Χρήστος Κουτσάκης, Τμήμα Χημείας, Πανεπιστήμιο Πατρών

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Αθανάσιος (Νάσος)-Νασίρ Σόκατ, Τμήμα Ιατρικής, Πανεπιστήμιο Πατρών

Αθανάσιος Χατζόπουλος, Τμήμα Χημείας, Πανεπιστήμιο Πατρών

Invited Lecturers

Panagiota Stefanopoulou, PhD
Key Account Manager, Life Science, Merck Greece

Juergen Luenzer, PhD
CEO Nippon Genetics Europe GmbH

Vasiliki Michopoulou, PhD
Journalist-Science writer, BS Biology, MA Political sciences, PhD International Relations, Teaching Fellow and Instructor at the School of Journalism and Mass Communications, Aristotle University of Thessaloniki, Greece

George N. Vlahakis, PhD
Associate Professor, School of Humanities, Hellenic Open University, Patras, Greece; Director of Postgraduate Program on Science Communication, Hellenic Open University, Patras, Greece

Charalampos Mentis, PhD
Scientific Officer at H.F.R.I. (Hellenic Foundation for Research and Innovation)

Michaela Filiou, PhD
Assistant Professor of Biochemistry, Department of Biological Applications and Technology, University of Ioannina; Affiliated Researcher, BRI-FORTH; Greek National L'Oréal-UNESCO 2021 Awardee

Nikolaos Afratis, PhD
Assistant Professor, Department of Agricultural Development, Agrofood and Management of Natural Resources, National and Kapodistrian University of Athens

Agenda

09:00 – 09:30 Registration – Welcome Addresses

SELECTED TALKS (I)

Chair: C. Koutsakis, Z. Piperigkou

09:30 – 10:20 ST1-ST5

ST1 **C. Katsioulas** *(IMBB-FORTH)*
Mechanistic insights into RNA-binding protein interactions that affect long non-coding RNA chromatin association

ST2 **M. Deiktakis** (*University of Crete*)
Evaluation of metabolic outcomes of Crh-/- mice exposed to diet-induced obesity

ST3 **D. Amanatidou** *(International Hellenic University)*
In Vitro evaluation of compounds of *Thymus thracicus* for inhibition of PTP1b, involved in insulin receptor desensitization

ST4 **C. Zorzompokou** (*University of Ioannina*)
Activation of Homologous Recombination and Non-Homologous End Joining after DNA damage in mouse oocytes

ST5 **D. Bainanztou** (*University of Patras*)
Artificial bioscaffolds promote chondrogenesis in human Wharton's Jelly mesenchymal stem cells

SPECIAL SESSION (I)

10:20 – 11:00 Good Laboratory Practice – Protein Analysis

Chair: N. Giarimoglou, A.N. Shaukat

10:20 – 10:40 **P. Stefanopoulou** (*Life Science, Merck Greece*)
Enhance your cell and protein analysis with novel Merck instrumentation

10:40 – 11:00 **J. Luenzer** (*Nippon Genetics Europe GmbH*)
Protein Electrophoresis – history and new developments

11:00 – 11:20 Coffee Break

SELECTED TALKS (II)

Chair: M. Skipitari, P. Papadea

11:20 – 12:00 ST6-ST9

ST6 **C. Koutsakis** *(University of Patras)*
Evaluating the action of sulfated hyaluronan in conventional 2D cultures and 3D spheroids of breast cancer cells

ST7 **G.-M. Sagia** *(National and Kapodistrian University of Athens)*
In search of the molecular basis of Sec24 interaction with cargoes sorted in the plasma membrane via Golgi-bypass

ST8 **A. Fotopoulou** (*NCSR "Demokritos"*)
A subset of human skin fibroblasts exposed to UVB radiation can escape premature senescence

ST9 **K. Karamanou** (*NCSR "Demokritos"; Agricultural University of Athens*)
In vitro screening of extracts from the Greek flora as a basis for the development of innovative cosmeceuticals

12:00 – 14:00 Free Time

SPECIAL SESSION (II)

14:00 – 14:30 Science Communication
Chair: S. Bournaka, V. Savvopoulou
14:00 – 14:15 **V. Michopoulou** *(Journalist-Science writer, Aristotle University of Thessaloniki)*
Keep it short and simple
14:15 – 14:30 **G.N. Vlahakis** (*Hellenic Open University)*
Science Communication as a vehicle for understanding Life Sciences in the public sphere

14:30 – 16:00 **Poster Session** (P1-P58)

SELECTED TALKS (III)

Chair: M. Politi, A.N. Shaukat
16:00 – 17:10 ST10-ST16

ST10 **S. Kairis** *(University of Patras)*
Effects of Cannabidiol pre-treatment on behavioral, inflammatory and biochemical markers on male mice after Concanavalin-A intoxication

ST11 **E. Rakovoliou** *(BRI-FORTH)*
The role of ARF6 in human embryonic stem cell pluripotency and differentiation

ST12 **G. Ellinas** *(Biomedical Research Foundation of Academy of Athens*)
The role of placental CRH in human brain development

ST13 **S.-F. Saravanou** *(University of Patras)*
Thermoresponsive bioink of sodium alginate-based graft copolymers for spontaneous cell-spheroids formation and growth

ST14 **P. Georgiopoulou** *(University of Patras)*
Investigating COVID-19 severity with NMR metabolomic analysis

ST15 **S. Notopoulou** *(CERTH)*
Proteomics analysis reveals key regulators of Spinocerebellar Ataxia Type 1 molecular pathology

ST16 **C. Ntallis** *(University of Patras)*
Use of Structure-Based Virtual Screening for the identification of novel allosteric inhibitors that target the Thioredoxin Reductase (TrxR) of *Escherichia coli*

17:10 – 17:20 Coffee Break

SPECIAL SESSION (III)

17:20 – 18:05 Career Choices & Advice
Chair: A. Chatzopoulos, Z. Piperigkou
C. Mentis *(Hellenic Foundation for Research and Innovation)*
H.F.R.I. as the main pillar for blue-sky research. Focusing on Biochemistry & Molecular Biology
M. Filiou *(University of Ioannina)*
Getting funded in science: Tricks and tips
N. Afratis *(National and Kapodistrian University of Athens)*
Career opportunities beyond the lab bench

18:05 – 19:00 **Discussion Groups & Networking**

19:00 – 19:30 **Awards & Closing Remarks**

SHORT TALKS

ST1

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

Christos Katsioulas, Evgenia Ntini*

Institute of Molecular Biology and Biotechnology of the Foundation for Research and Technology Hellas, Heraklion, Greece
*Corresponding author

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

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

ST2

Evaluation of metabolic outcomes of Crh-/- mice exposed to diet-induced obesity

Michail Deiktakis, Ourania Kolliniati, Efstathia Lefkothea Papadiamanti, Chrysi Mouatsou, Christos Tsatsanis, Andrew N. Margioris, Maria Venihaki, Eirini Dermitzaki*

Lab of Clinical Chemistry, School of Medicine, University of Crete, Heraklion, Greece

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

neuropeptide for the activation of HPA axis, the aim of the present study was to investigate the metabolic effects of diet-induced obesity in mice lacking CRH (Crh-/-).
Materials-Methods
Crh-/- and wild-type (Crh+/+) mice were fed with high-fat (HFD45%) or standard-chow (SC) diet for 13 weeks. Weight-change and food-intake were measured weakly. Blood glucose levels were measured in the fasting state before and after the 13-weeks' feeding. Glucose-tolerance-test (GTT) was performed via i.p. dextrose injection after the 13-weeks period. Mice were sacrificed and White-Adipose-Tissue (WAT) was collected for qPCR analysis.
Results
Fasting blood glucose levels were dramatically increased in HFD45%-fed compared to SC-fed mice regardless of genotype. Wild-type mice gained significantly more weight when fed with HFD45% compared to Crh-/- mice although the amount of HFD45% food-intake was the same between genotypes. Additionally, Crh-/- mice were capable of faster glucose clearance measured by GTT than wild-type mice in the HFD45% group. Interestingly, leptin mRNA levels were significantly less in WAT of Crh-/- mice compared to wild-type mice in the HFD45% group.
Conclusions
Our results provide evidence that Crh-/- mice have improved metabolic outcomes when exposed to diet-induced obesity. Interestingly, the Crh-/- mice exhibited reduced weight gain and improved glucose homeostasis under diet-induced obesity compared to wild-type mice. Our ongoing analyses attempt to further elucidate the metabolic mechanisms of this phenomenon.

ST3

In vitro **evaluation of compounds of** ***Thymus thracicus*** **for inhibition of PTP1b, involved in insulin receptor desensitization**

Dionysia Amanatidou[1], Theodora Papagrigoriou[2], Diamanto Lazari[2], Phaedra Eleftheriou[1]

[1]Department of Biomedical Sciences, School of Health, International Hellenic University, Thessaloniki, Greece
[2]Department of Pharmacognosy-Pharmacology, School of Pharmacy, Faculty of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, Greece.

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

References
1. Dessalegn E. et al. Evaluation of In vitro Antidiabetic Potential of *Thymus schimperi R.* and *Thymus vulgaris L.* Journal of Health, Medicine and Nursing, 69, 9-16, 2019.
2. Haile T, Cardoso SM et al. Chemical Composition, Antioxidant Potential, and Blood Glucose Lowering Effect of Aqueous Extract and Essential Oil of *Thymus serrulatus* Hochst. Ex Benth. Front. Pharmacol. 12, 621536, 2021.
3. Azhar J, John P., Bhatti A. *Thymus serpyllum* Exhibits Anti-Diabetic Potential in Streptozotocin-Induced Diabetes Mellitus Type 2 Mice: A Combined Biochemical and In Vivo Study. Nutrients,14, 3561, 2022.
4. Bing TZ et al. Protein tyrosine phosphatase 1B inhibitors from natural sources. Arch. Pharm. Res. 41:130–161, 2018.
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6. Papagrigoriou T. Pharmacognostic study of the *Thymus thracicus* Velen. Plant. Master thesis, Thessaloniki, 2019.

ST4

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

Chysoula Zorzompokou[1,2]*, Marios Ipeirotis[1,2], Petros Marangos[1,2]

[1]Laboratory of Cell and Developmental Biology, Department of Biological Applications and Technology, University of Ioannina, Ioannina, Greece
[2]Biomedical Research Institute, Foundation for Research and Technology-Hellas, Ioannina, Greece
Correspondence: ch.zorzobokou@uoi.gr

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

ST5

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

Dimitra Bainantzou[1], Nadia Makri[1], Zoi Piperigkou[1,2], Dimitra Manou[1], Paraskevas Lamprou[3], Rigini Papi[3], Theodora Choli-Papadopoulou[3], Nikos K. Karamanos[1,2], Achilleas D. Theocharis[1*]

[1]Biochemistry, Biochemical Analysis & Matrix Pathobiology Research Group, Laboratory of Biochemistry, Department of Chemistry, University of Patras, Greece
[2]Foundation for Research and Technology-Hellas (FORTH)/Institute of Chemical Engineering Sciences (ICE-HT), Patras, Greece
[3]Laboratory of Biochemistry, School of Chemistry, Aristotle University of Thessaloniki, Greece
*Corresponding author

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

This work has been funded by the project ArthroMicroPerMed (MIS 5033644), implemented under the "Action for the Strategic Development on the Research and Technological Sector", 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).

ST6

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

Christos Koutsakis[1], Zoi Piperigkou[1,2], Nikos K. Karamanos[1,2]

[1]Biochemistry, Biochemical Analysis & Matrix Pathobiology Research Group, Laboratory of Biochemistry, Department of Chemistry, University of Patras, Greece
[2]Foundation for Research and Technology-Hellas (FORTH) / Institute of Chemical Engineering Sciences (ICE-HT), Patras, Greece

Breast cancer constitutes one of the most common malignancies, with the expression patterns of estrogen receptors (ERs) being crucial during disease development. Hyaluronan (HA) is an extracellular matrix glycosaminoglycan (GAG) that plays a central role in a variety of biological processes. While HA

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

Sulfated HA was kindly provided by Fidia Farmaceutici S.p., (Abano Terme, Italy).

ST7

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

Georgia Maria Sagia[1], Mariangela Dionysopoulou[1], Vasilis Koudounis[1], Sofia Dimou[1] and 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 and pack into COPII secretory vesicles, which are destined to fuse with the cis-Golgi. Following Golgi maturation, membrane proteins exit from the trans-Golgi network in clathrin coated vesicles directed to the PM1. However, our recent findings in *Aspergillus nidulans* showed that several transmembrane cargoes follow an 'unconventional' sorting pathway that bypasses the Golgi2,3, which in turn suggests the existence of distinct cargo-specific COPII subpopulations. Selective recruitment of membrane cargoes in COPII by Sec24 is mediated by direct interactions between cargo-binding sites in Sec24 and specific sorting motifs in cargoes4,5. Here we investigate the molecular basis of Sec24 interactions with the UapA transporter, a paradigmatic Golgi bypasser. For this, we develop a controllable system to repress the synthesis of the endogenous Sec24 and use it to investigate the functional effect of systematically designed Sec24 mutations carried in a plasmid vector introduced by reverse genetics. In parallel, we re-investigate the role of a cytoplasmically located N-terminal motif of UapA in ER-exit and PM localization. Our results show that i) amino acid substitutions in conserved or semi-conserved residues located in the A and B cargo-binding sites of Sec24 are critical for UapA ER-exit and/or fungal growth ii) a Tyr residue in the N-terminal motif of UapA is essential for ER-exit and seemingly crucial for recognition by Sec24.

References
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ST8

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

Asimina Fotopoulou[#], Maria Angelopoulou[#], Harris Pratsinis, Eleni Mavrogonatou and Dimitris Kletsas*

Laboratory of Cell Proliferation and Ageing, Institute of Biosciences and Applications, National Centre for Scientific Research "Demokritos", 15341, Aghia Paraskevi, Attiki, Greece
*Corresponding author

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

ST9

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

Konstantina Karamanou[1], Asimina Fotopoulou[1], Gabriela Belen Lemus Ringele[2], Eleftherios Kalpoutzakis[2], Aikaterini Argyropoulou[3], Eleni Mavrogonatou[1], Georgios Stavropoulos[4], Maria Halabalaki[2], Harris Pratsinis[1], Dimitris Kletsas[1,*]
[1]Laboratory of Cell Proliferation and Ageing, Institute of Biosciences and Applications, NCSR "Demokritos", Athens
[2]Division of Pharmacognosy and Natural Products Chemistry, Department of Pharmacy, National and Kapodistrian University of Athens, Athens
[3]PharmaGnose S.A., Oinofyta, Boeotia
[4]KORRES Natural Products S.A., Metamorfosi, Athens
*Corresponding author

The unique biodiversity of the Greek flora– including a considerable percentage of endemic plant taxa – has triggered an increasing interest in the identification of bioactive compounds originating from these plants. We have focused especially on natural products with the ability to counteract one or more of the features of skin ageing, such as extracellular matrix degradation, increased reactive oxygen species, hyperpigmentation etc. The present work is part of a collaborative project that will eventually screen 104 plant extracts from different areas of Greece for biological activities necessary for the development of innovative cosmeceuticals. The cytotoxic activities of the above extracts against human skin fibroblasts and keratinocytes have been assessed using two methodologies, the MTT and the Neutral Red assays, in order to identify the highest non-cytotoxic concentrations for further analyses. In parallel, the putative activities of the extracts against hyperpigmentation and against elastin degradation, were assessed *in vitro* using photometric assays and utilizing mushroom tyrosinase and porcine pancreas elastase, respectively. The majority of the extracts were not cytotoxic at all (up to 100 μg/ml) for human skin fibroblasts and HaCaT keratinocytes (approximately 61% and 85% of the extracts for each cell type, respectively). For all other extracts the highest non-cytotoxic concentrations were determined ranging from 20 to 0.8 μg/ml. Regarding tyrosinase activity, it was found to be inhibited at over 50% by 15 out of the 104 extracts tested. Moreover, 20 out of 104 extracts were found to inhibit elastase activity by 50% or more. Some extracts were sharing both anti-tyrosinase and anti-elastase activities. In conclusion, this initial biological profiling of extracts from the Greek flora indicates that they could support the development of novel and high-quality cosmetics.

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ST10

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

Stefanos Kairis[1], Korina Atsopardi[1,2], Konstantinos Mesiakaris[2], Nikolaos T. Panagopoulos[1], Marigoula Margarity[1], Konstantinos Poulas[2]

[1]Laboratory of Human and Animal Physiology, Department of Biology, University of Patras, Greece
[2]Laboratory of Molecular Biology and Immunology, Department of Pharmacy, University of Patras, Greece

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

Con-A intoxication (inflammation index) and were reduced when mice were pre-treated with CBD. The results indicate a decrease in AChE activity in the cerebral cortex, striatum and diencephalon in both G1 and G4 isoforms in the CBD group but also in the Con-A group as well as in the co-administration of Con-A and CBD.

ST11

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

Elena Rakovoliou[1], Angelos Papadopoulos[2], Maria Markou[1], Sofia Bellou[1,3], Nikoleta Kostopoulou[1], Eleni Bagli[1], Theodore Fotsis[1,4] and Carol Murphy[1]*

[1]Foundation of Research and Technology-Hellas, Biomedical Research Institute, University Campus, 45110 Ioannina, Greece
[2]School of Biosciences, College of Life and Environmental Sciences, University of Birmingham, UK.
[3]Confocal Laser Scanning Microscopy Unit, Network of Research Supporting Laboratories, University of Ioannina, Ioannina, 45110, Greece.
[4]Laboratory of Biological Chemistry, Medical School, University of Ioannina, 45110 Ioannina, Greece
*Corresponding author, email: carol_murphy@bri.forth.gr

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

ST12

The role of placental CRH in human brain development

George Ellinas[1], Georgia Kouroupi[2], Rebecca Matsas[2], Panagiotis K. Politis[1], Ioannis Serafimidis[1]and Yassemi Koutmani[1]*

[1]Biomedical Research Foundation of Academy of Athens, Athens, Greece
[2]Hellenic Pasteur Institute, Athens, Greece

Among mammals, anthropoid primates display a unique expression pattern of feto-maternal hormonal interaction. During pregnancy, their placenta is progressively producing enormous amounts of the Corticotropin-Releasing Hormone (CRH). Although the role of hypothalamic CRH has been extensively studied in the physiology of stress, there is a remarkable lack of evidence regarding the role of placental CRH, while the biological significance of its unique expression pattern in anthropoid primate species remains elusive.
In order to investigate the effects of placental CRH on human brain development and to overcome the limitations raised in experimenting with human tissue, we have generated human 3D-neural spheroids and human cerebral cortical organoids from human embryonic stem cells (hESCs). Exposure of neural spheroids to CRH during a 50 days culture period, results in significant differences in the size and the cellular composition of the spheroids as assessed by imunohistochemical detection of specific markers of the neural lineage. Pharmacological disruption of the CRH signaling reverses the effects of CRH in the same culture model. We also revealed altered cellular fate, gene expression and architectural characteristics in 50 days human cerebral cortical organoids.
The key role of CRH in stress physiology and the human-specific pattern of placental CRH expression, suggest that this *in vitro* approach provides a unique tool for our understanding of the mechanisms underlying the role of stress hormones in human physiology and raises the intriguing possibility for pharmacological applications in neurodevelopmental disorders and deficits associated with anxiety.

ST13

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

Sofia-Falia Saravanou[1], Thomai Samouilidou[2], George Pasparakis[1], Stavros Taraviras[2]

[1]Department of Chemical Engineering, University of Patras, 26500 Patras, Greece
[2]Department of Physiology, School of Medicine, University of Patras, 26500 Patras, Greece

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

ST14

Investigating COVID-19 severity with NMR metabolomic analysis

Panagiota D. Georgiopoulou[1], Georgios Schinas[2], Styliani A. Chasapi[1], Eleni Polyzou[2], Karolina Akinosoglou[2]*, Georgios A. Spyroulias[1]*

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

[2] Department of Medicine, University of Patras, 26504 Patras, Greece
*Equal co-authorship

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

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ST15

Proteomics analysis reveals key regulators of Spinocerebellar Ataxia Type 1 molecular pathology

Sofia Notopoulou, Spyros Petrakis

Institute of Applied Biosciences, CERTH, Thessaloniki, Greece

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

ST16

Use of Structure-Based Virtual Screening for the identification of novel allosteric inhibitors that target the Thioredoxin Reductase (TrxR) of *Escherichia coli*

Charalampos Ntallis[1], Ioannis Georgantas[2], Alexios Vlamis-Gardikas[1,*]

[1]Department of Chemistry, University of Patras, Greece
[2]Institute of Chemical Engineering Sciences, Foundation for Research and Technology, Greece
*Correspondence: avlamis@upatras.gr; Tel.: +30-2610-997-634

The thioredoxin (Trx) system, along with the glutathione based glutaredoxin system, channel the reducing antioxidant electron flow to the cytosol of most living cells including bacteria and viruses. The two systems may regulate a plethora of additional cellular processes such as DNA synthesis and repair, and the activation of redox-dependent transcription factors. In the Trx system, electrons are sequentially transferred from NADPH to thioredoxin reductase (TrxR), Trx and finally to the substrates of the latter. Differences in the structure and catalytic mechanisms between mammalian and bacterial TrxRs render the bacterial enzymes as potential drug targets. In the present study, Structure-Based Virtual Screening (SBVS) was performed for the discovery of novel allosteric inhibitors that may selectively target the TrxR of Escherichia coli (EcTrxR). To achieve maximum selectivity of the hit compounds, we used a crystal structure of the oxidized form of EcTrxR (PDB ID: 1TDE), where we implemented a straight-forward SBVS protocol. With the use of the MCULE online platform, molecular docking was performed with the Autodock Vina algorithm for the ~9 million compounds marked as "in stock" in the MCULE database. Based on the docking score, the 1000 top-ranking compounds were selected and subjected to toxicity and drug-likeness filters. Next, further validation was performed by comparing our results to the molecular docking properties of known inhibitors of EcTrxR with IC50 values taken from the ChEMBL database (e.g., ebselen). Ten top hit compounds were finally selected after close inspection of their interactions with the residues of the active site (hydrogen bonding, salt bridges and van der Waals interactions). The selected compounds appear as good candidates for further investigation as antimicrobials.

POSTERS

P1

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

Kostis Konstantinidis[1], Eva Papastergiadou[2], Yiannis Vasilopoulos[1]*

[1]Laboratory of Genetics, Section of Genetics, Cell Biology and Development, Department of Biology, University of Patras, 26504 Patras, Greece
[2]Section of Plant Biology, Plant Ecology & Management of Freshwater Ecosystems, Department of Biology, University of Patras, 26504 Patras, Greece
*Email: iovasilop@upatras.gr

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

conservative region of gene matK. Results showed positive amplification only with universal primers, indicated that *Chara hispida f. corfuensis* is not present in the particular habitat of the water sampling station.

P2

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

Despoina Eugenia Kiousi[1], Christos Efstathiou[1], Vasilis Tzampazlis[1], Maria Panopoulou[2], Maria Koffa[1] and Alex Galanis[1]*

[1]Department of Molecular Biology and Genetics, Faculty of Health Sciences, Democritus University of Thrace, Alexandroupolis, Greece
[2]Department of Medicine, Faculty of Health Sciences, Democritus University of Thrace, Alexandroupolis, Greece
*Correspondence to: agalanis@mbg.duth.gr

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

P3

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

Ioanna Lapi[1,2]*, Ioanna Pantazi[1,2], Eleni Paflioti[1,2], Stergios Chatzisevastos[1,2], Ismini Marava[1,2], Evangelia Kandylaki[1,2], Theoni Maria Michalopoulou[1,2], Maria Venihaki[1], Christos Tsatsanis[1,2]

[1]Laboratory of Clinical Chemistry, Medical School, University of Crete, 70013 Heraklion, Greece.
[2]Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology Hellas, 71100 Heraklion, Greece.
*Email: ioanna.lapi@outlook.com

Wound healing is a dynamic and complex process involving four interconnected stages, in which chemokines are key orchestrators. Nutrition is an important factor that directly affects skin homeostasis, whereas malnutrition impairs tissue healing. Every year, a large number of sidestreams are produced by the fish processing industry, which are either discarded or underutilized. This raw material, which contains a great amount of muscle and connective tissue with high protein content, can be subjected to enzymatic hydrolysis to generate protein hydrolysates, a source of bioactive peptides. In addition to their biological activity, many of these marine derivatives also have great nutritional value; therefore, a beneficial effect on human health could emerge.
In the present study, we used fish sidestream-derived protein hydrolysates including fish collagen as dietary supplements to test their impact on the skin repair process using an *in vivo* cutaneous wound healing model. We explored potential differences in wound closure and histological morphology between the diet groups and analyzed the expression and production of factors that participate in different stages of the repair process.
Dietary supplementation with fish sidestream-derived collagen alone (Collagen), or in combination with a protein hydrolysate derived from salmon heads (HSH), resulted in accelerated healing. Tissues from mice fed with collagen-containing supplements exhibited an increase in the expression levels of chemokines, important for the recruitment of immune cells into the damaged wound region. According to the chemical analysis, Collagen had the highest protein content and HSH contained a large amount of zinc, which is known to support immune responses. Our findings suggest that a 5%-supplemented diet with marine collagen-enriched supplements promotes tissue repair in the model of cutaneous wound healing, proposing a novel health-promoting use of fish sidestreams.

P4

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

Athanasios Chatzopoulos[1#], Theodoros Karalis[2#], Nikos K. Karamanos[1], Paraskevi Heldin[2], Spyros S. Skandalis[1*]

[1]Biochemistry, Biochemical Analysis & Matrix Pathobiology Res. Group, Laboratory of Biochemistry, Dept. of Chemistry, University of Patras, Greece
[2]Dept. of Medical Biochemistry and Microbiology, Uppsala University, Sweden
#equal contribution
*skandalis@upatras.gr

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

resulted in dephosphorylation of rpS6 in agreement with the hyaluronan biosynthesis inhibition results. These findings indicate a critical regulatory role for hyaluronan in metastatic breast cancer cell growth.

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P5

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

Athanasios A. Panagiotopoulos, Konstantina Kalyvianaki, Evangelia Konstantinou, George Notas, Elias Castanas*, Marilena Kampa*

Laboratory of Experimental Endocrinology, University of Crete, School of Medicine, Heraklion, Greece
*Emails: kampam@uoc.gr; castanas@uoc.gr

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

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P6

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

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

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

Melanoma is the most aggressive type of skin cancer, characterized by high metastatic potential and its development is affected by the presence of both hereditary and environmental factors. The most common mutation in melanoma is BRAFV600E, which leads to excessive downstream signaling and translational deregulation. Targeting of BRAFV600E using specific small molecule inhibitors, such as vemurafenib, is widely used for treatment of metastatic melanoma. However, malignant cells rapidly develop resistance to targeted therapy. eIF6 was recently reported as a promising diagnostic and prognostic biomarker for poorer survival of melanoma. Possessing both ribosomal anti-association and 60S-biogenesis roles, eIF6 is overexpressed in many cancer types. Participation of eIF6 in the development and acquired resistance of melanoma cells remains to be elucidated. In the present study, we developed BRAFV600E-mutated vemurafenib resistant (VR) A375 and SK-MEL5 cell lines. eIF6 was differentially expressed in the VR cells and its nucleus/cytoplasm localization ratio was altered. The 60S:40S, though, was not affected, as indicated by ribosome profiling using sucrose density gradients. To further investigate its role in translation regulation and signaling in melanoma, we constructed eIF6-stably expressing A375 and SK-MEL5 cell lines. eIF6-overexpressing A375 cells, mainly showed a slight decrease in the phosphorylation of S6 kinase, while metastatic SK-MEL5 cells showed a significant increase in the phosphorylated forms of AKT, S6K and ERK kinases and a decrease of the phospho-mTOR (S2448) kinase. Both cell lines showed higher expression of total and phospho-eIF4E, indicating enhanced cap-dependent translation. Puromycin staining revealed increased global translation rates only in eIF6-overexpressing A375 cells. Basal levels of eIF6 were also checked in four genetically engineered mouse melanoma cells lines, showing increased expression of eIF6 in two of them. Collectively, our results suggest a significant role of eIF6 in both vemurafenib resistant melanoma cell lines by affecting major signaling events and cap-dependent translation regulation.

P7

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

Evangelia Voulgaraki[1,2], Eirini Taouktsi[1,2], Eleni Kyriakou[1], Popi Syntichaki[1*]

[1]Biomedical Research Foundation of the Academy of Athens, Laboratory of Molecular Genetics of Aging, Center of Basic Research, Greece
[2]Agricultural University of Athens, Greece
*Email: synticha@bioacademy.gr

Mitochondrial damage leads to accumulation of misfolded/unfolded proteins and activation of stress responsive pathways to cope with proteotoxic stress. The highly conserved ATP-dependent mitochondrial LONP1 protease is an important regulator of mitochondrial protein homeostasis, acting both as a chaperone and a protease. We employed here a null mutant *Caenorhabditis elegans* to study how deficiency of the lonp-1 gene affects the organismal responses in the nematode. We have shown that LONP-1 deficiency results in short-lived animals with disturbed mitochondrial network and excessive ROS production. These defects initiate an adaptive cellular signaling and induce the Antioxidant Stress Response, the Heat Shock Response and the mitochondrial Unfolded Protein Response (UPRmt)1. Under conditions of mitochondrial stress, UPRmt activity normally restricts the activation of a p38/MAPK (MAPKmt) mitochondrial surveillance pathway, consisting of the DLK-1/SEK-3/PMK-3 signaling cascade2,3. Induction of MAPKmt under mitochondrial respiratory dysfunction is triggered downstream

of the Mitochondrial Associated Degradation (MAD) pathway3. The MAD pathway mediates export of ubiquitinated proteins, through the CDC-48/UFD-1/NPL-4 protein complex of the Mitochondrial Outer Membrane (MOM) and transfers them to the proteasome for degradation4. These molecular interactions indicate the presence of extensive crosstalk between cellular surveillance mechanisms following mitochondrial stress. To gain insight into these quality control mechanisms, we investigated the impact of LONP-1 deficiency on the activation of and interplay between the associated pathways. In this study, we have demonstrated that the MAPKmt pathway is induced in lonp-1(ko) mutants, even though they exhibit increased activity of ATFS-1, a major transcription factor of UPRmt. However, knock down of atfs-1, further upregulates MAPKmt, supporting the restrictive role of UPRmt on MAPKmt induction. Furthermore, we currently focus on the interaction of MAD and MAPKmt pathways in lonp-1(ko) mutants, after silencing key components of the MAD pathway in transgenic animals expressing an established marker of MAPKmt cascade.

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P8

Blocking Nanobodies as potential "safety switches" in T-lymphocytes

Ioannis Tyritidis*, Pantelis Christou, Nikos Koutras, Kyriakos Konnaris, Konstantina Nika

Department of Biochemistry, School of Medicine, University of Patras, Patras, Greece

The selective and controllable inhibition of T-cell function is crucial not only for the design of personalized treatments against T-cell-dependent pathologies, but it can also comprise an invaluable tool in the fields of organ transplantation and T-cell immunotherapies. Hence intense efforts are focused on identifying compounds capable of manipulating T-cell activation. Lck, a member of the Src family of protein tyrosine kinases (SFKs), is mandatory for T-cell signaling initiation and subsequently T-cell activation and has become an attractive target for the production of small molecule inhibitors. However, the development of highly selective and potent Lck inhibitors has not been met with success, due to an astonishing structural homology shared by SFK members within their catalytic centers. The current project aims to achieve selective downregulation of T-cell signaling and T-cell function via specific inhibition of Lck, using intracellularly expressed nanobodies (Nbs).
These Nbs are designed to recognize a poorly conserved regulatory region of Lck and will act in endosomal compartments in order to prevent the translocation of Lck into the plasma membrane and immunological synapse, the natural locations of Lck activity. This alteration in Lck traffic should block the initiation of T-cell signaling and downregulate T-cell function.
A preliminary screening of different Nbs was accomplished by transfecting HEK293T cells with Lck in the presence or absence of Nbs. The Nbs that were capable to bind Lck were further transduced in T-cell lines, and it was tested the ability of Nbs to create a binding complex with the endogenous Lck, using Co-Immunoprecipitation experiments and confocal microscopy.

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

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P9

McIdas cell cycle regulation is necessary for genome integrity

Spyridoula Bournaka[1], Marina Arbi[1], Stavroula Tsaridou[1], Vicky Petroulaki[1], Elena Karydi[1], Stavros Taraviras[2], Zoi Lygerou[1*]

[1]Laboratory of Biology, School of Medicine, University of Patras, Greece
[2]Laboratory of Physiology, School of Medicine, University of Patras, Greece
*Email: z_lygerou@yahoo.com

Maintaining the balance between proliferation and differentiation is crucial for multicellular organisms and this is achieved through the orchestration of factors that control these processes. Geminin superfamily members -Geminin, McIdas and GemC1- is a group of distantly related coiled-coil proteins characterized for their roles both in DNA replication initiation and multiciliated cell differentiation1. Here, we describe how McIdas is regulated during the cell cycle and the impact of its deregulation on genome integrity. The levels of McIdas drop after anaphase and increase again before S phase. McIdas is an APC/C substrate, and it is recognized through two destruction sequencies, DBox and ABBA motifs. A mutational analysis on McIdas destruction sequences was performed and we showed that the cell cycle profile of the cells was unaffected. However, the non-degradable forms of McIdas led to genomic instability phenotypes, such as DNA bridges, micronuclei, multinuclear cells and 53BP1 nuclear bodies. These phenotypes are linked to DNA replication perturbation, illustrating an important role for McIdas in genome duplication2. The members of the Geminin superfamily are involved in an increased number of cellular processes, therefore understanding their functions will allow their role in the balance between proliferation and differentiation to be elucidated.

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P10

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

Maria S. Manola*, Alexandra Drakaki, Sentiljana Gumeni, Ioannis P. Trougakos

Department of Cell Biology and Biophysics, Faculty of Biology, National and Kapodistrian University of Athens, Greece
Age-associated phenotypes are mainly caused by the progressive accumulation of biomolecular damage that along with the gradual declining capacity of cellular stress response mechanisms lead to the

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

P11

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

Niki Fasitsa, Eleni Nteli, Sofia Marka, Maria- Eleni Zografaki, Konstantina Karamanou, Anastasia- Marina Palaiogeorgou, Sophie Mavrikou*, Spyridon Kintzios

Laboratory of Cell Technology, Department of Biotechnology, Agricultural University of Athens, EU-CONEXUS European University, Athens, 11855, Greece

Ailanthus altissima is an invasive species of the Simaroubaceae family, that has been introduced to the native flora of Greece. The fruits of *Ailanthus altissima* contain several bioactive compounds which may contribute to the development of new anticancer agents with potential applications in the cosmetics or pharmaceutical industry. Thus, in this study plant extracts were screened for their antioxidant, antimicrobial and cytototoxic properties. To prepare the extracts, leaves and barks were lyophilized, underwent tissue breakage, and the ethanolic and methanolic extracts were performed by using maceration, ultracentrifugation, and heat- and ultrasound-assisted extraction (HAE and UAE). *Ailanthus altissima* extracts were assessed for their antioxidant activity. The assays employed were (FRAP), ferric reducing antioxidant power, and scavenging effect on the 1,1-diphenyl-2-picrylhydrazyl free radical (DPPH). *Ailanthus altissima* extracts revealed significant effects in DPPH scavenging as well as hydroxyl radical scavenging activity and ferrous ions-chelating ability. The medicinal plants are known to contain number of phenolic compounds with strong antioxidant activity. Therefore, the total phenolic contents of the plant extracts were also determined spectrophotometrically according to Folin- Ciocalteau method. Moreover, in this study, *in vitro* toxicological assessment of the *ailanthus altissima* extracts was performed in the human neuroblastoma cell line SK-N-SH. *Ailanthus altissima* extracts inhibited cancer cell proliferation and displayed cytotoxic activity in SK-N-SH neuroblastoma cells. The extracts of Ailanthus altissima exhibited also *in vitro* antimicrobial activity by agar disk diffusion method and MTT cytotoxic assay against *Klebsiella oxytoca* and *Debaryomyces spp*. This work provides scientific supports for the high antioxidant and phytotoxic activities of this species and thus, it may find potential applications in the development of natural herbicides and antioxidants for agro-food and pharmaceutical industries.

P12

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

Dafni-Alexandra Kavvoura[1], Michalis K. Stefanakis[2], Dimitris Kletsas[1], Haralambos E. Katerinopoulos[2], Harris Pratsinis[1, *]

[1]Laboratory of Cell Proliferation and Ageing, Institute of Biosciences and Applications, NCSR "Demokritos", Athens
[2]Laboratory of Organic Chemistry, Department of Chemistry, University of Crete, Voutes, Heraklion, Crete

Carob tree (*Ceratonia siliqua*) is an evergreen tree of the Fabaceae family mainly met in the Mediterranean Basin. The nutritional value of carob is well known since ancient times, though somewhat neglected nowadays, in spite of the fact that it played an important role in the survival of the Cretans and the Spanish during the second world war and the civil war, respectively. Moreover, various parts of the carob tree may be useful in terms of their medicinal properties. Hence, there is a revived interest for carob tree cultivation, especially in warm Mediterranean regions like Crete. Since, literature data suggest that some activities of carob extracts are strongly influenced by gender and cultivar, our aim was to characterize samples from various *Cretan Ceratonia siliqua cultivars* in terms of their chemotaxonomic and bioactivity properties. The initial chemical characterization of a cultivar originating from the area of Elounda (Eastern Crete) was based in the identification of secondary metabolites following cold extraction in either methanol/water or hexane and using NMR and ESI-MS. Clevenger distillation followed by GC/MS,analysis revealed the presence of twenty major volatile components including α-(Z,E)-farnesene, heptadecane, nonadecane, hexadecanoic acid, octadecanoic acid and E-phytol acetate. Compounds such as sucrose, glucose, fructose, pinitol and myo-inositol were identified and quantified in the extracts. Moreover, some of the extracts were found to possess selective cytostatic activity against cancer cell lines, as well as, significant antioxidant activity both in cell-free and in cell-based assay systems. Finally, activities related to skin ageing inhibition were also identified. In conclusion, the chemical constituents and the biological properties identified in carob samples of Cretan origin are in line with literature reports concerning carob samples from other countries. Our aspiration regarding this ongoing effort is to provide chemotaxonomic and bioactivity data serving as a signature of each *Cretan Ceratonia siliqua cultivar*.

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P13

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

Dafni Doulaptsi Teeuwen, Sofia Veloudou, Konstantina Karpouzou, Vaggelis Tsioupros, Artemis Filippidou, Sotiris Tsakas, Ourania Pavlou, Irene Margiolaki and Eleftheria Rosmaraki

Division of Genetics, Cell and Developmental Biology, Department of Biology, University of Patras, Patras, Greece

Insulin injections are vital for millions of diabetic patients, improving their lives vastly. Microcrystalline insulin formulations are commonly used due to the stability and prolonged action they attribute; however, inflammation is a common phenomenon occurring at the infusion site of insulin in many patients. Since the underlying mechanisms for acute skin irritation and inflammation are unknown, we aimed to examine the effects of the organic ligands, m-cresol, p-coumaric acid, 4-ethylresorcinol, 4-chloro-resorcinol and 4-bromo-resorcinol, on the viability of human white blood cells, on neutrophil phagocytosis, and on cell migration. The ligands were selected due to the desired crystal properties they attribute to insulin and the potential future use for the development of new insulin formulations. These molecules, which were used in concentrations ranging from 50 to 1250 μM, exhibited mild differential effects on viability, phagocytosis and cell migration. To conclude, further experiments assessing the effects of these ligands

on phenomena closely associated with inflammation, such as the release of the pro-inflammatory cytokines, and the production of reactive oxygen species, are required.

P14

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

Katerina Pliatsika[1], Ioannis Gkekas[1], Antonis Tsiailanis[2], Andreas Tzakos[2] and Spyros Petrakis[1]

[1]Institute of Applied Biosciences, Centre for Research and Technology Hellas, Thessaloniki, Greece
[2]Chemistry Department, University of Ioannina, Greece

Spinocerebellar ataxia 1 (SCA1) is a fatal neurodegenerative disorder, which belongs to the group of polyglutamine (polyQ) diseases. SCA1 is caused by trinucleotide (CAG) repeat expansions in the ATXN1 gene and is associated with an expanded polyglutamine tract in the ataxin-1 protein. The expansion of CAG repeats in the ATXN1 gene alters the conformation of the protein and leads to its misfolding and self-assembly into insoluble inclusions. Protein inclusions are mainly found in the Purkinje cells of the cerebellum; however, they are thought to be responsible for the widespread neuropathology of SCA1 disease. We have previously shown that insoluble inclusions induce oxidative and nucleolar stress. PolyQ inclusions contain fibrillar β-sheets potentially formed through polar zippers, which eventually assemble into amyloid fibrils. Therefore, the development of sensitive methods for the detection of protein aggregates may contribute to the early diagnosis of SCA1 and the development of therapeutic approaches against polyQ-induced oxidative stress.
The aim of this study is the detection of β-amyloids in polyQ ataxin-1 inclusions. To this end, we generated inducible Tet-On YFP-ATXN1 (Q82) SH-SY5Y cells which accumulate polyQ inclusions and may be detected using β-amyloid-specific Near-InfraRed probes. We found that some of these probes detect amyloids compared to control SHSY5Y cells that do not contain protein inclusions. These results were further confirmed in assays using purified polyQ inclusions. This methodology may allow the monitoring of ataxin-1 aggregation which is responsible for the cellular oxidative stress.

P15

Investigating the role of Abracl in a neuronal cancer cell line

Imren Alioglu[1], Electra Stylianopoulou, Lydia Aggelopoulou[2], Venetia Giourou[1], Konstantinos Ntitsias[1], George Skavdis[2] and Maria E. Grigoriou[1]

[1]Laboratory of Developmental Biology & Molecular Neurobiology, Department of Molecular Biology & Genetics, Democritus University of Thrace
[2]Laboratory of Molecular Regulation & Development of Diagnostic Technology, Department of Molecular Biology & Genetics, Democritus University of Thrace, Alexandroupolis, Greece.

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

role in carcinogenesis. We are currently analyzing the effects of the downregulation of Abracl in knock out clones of Neuro2A cells generated by CRISPR/Cas9.

P16

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

Elisavet Ioannidou[1,2], Vagelis Rinotas[1], Panagiotis Theofilidis[1,2], Marili Skalioti[1,2], Trias Thireou[2], Eleni Douni[1,2]*

[1]Institute for Bioinnovation, Biomedical Sciences Research Center "Alexander Fleming", Vari, Greece
[2]Laboratory of Genetics, Department of Biotechnology, Agricultural University of Athens, Greece

Osteoporosis is a multifactorial disease characterized by bone loss, bone fragility and increased bone fracture risk and is often undiagnosed until the appearance of bone fractures. Our lab has established a genetic mouse model of osteoporosis by overexpression of human RANKL in transgenic mice (TgRANKL). In the current study, we identified differentially expressed (DE) genes with potential clinical value in osteoporosis by performing RNA-Seq for mRNAs, miRNAs, and lncRNAs in flushed femurs from TgRANKL and control wild-type (WT) mice. Selected genes were validated with qPCR in femurs from WT mice, untreated TgRANKL mice and treated with an anti-RANKL therapy (Denosumab). Regarding mRNAs, we identified in total 2,747 DE mRNAs (|log2FoldChangel > 1, adjusted p-value < 0.05), 959 of them being upregulated and 1,788 downregulated in TgRANKL femurs compared to WT. Enrichment analysis of the upregulated genes revealed that they were related to protein degradation, peptidase activity, and bone remodelling, while downregulated genes were related to oxidative phosphorylation, and metabolism. The expression of 39 confirmed upregulated genes returned to normal levels after Denosumab treatment, while it was examined in osteoclastogenesis and osteoblastogenesis experiments, too. Concerning miRNAs, we identified 63 DE miRNAs (|log2FoldChangel > 1, adjusted p-value < 0.05), 33 of them being upregulated and 30 downregulated. We validated with qPCR, 4 upregulated and 5 downregulated miRNAs. Regarding lncRNAs, we identified through RNA-Seq 235 DE lncRNAs (|log2FoldChangel > 2, adjusted p-value < 0.05), 80 of them being upregulated and 155 downregulated. The DE genes revealed in this study may serve as the basis for the discovery of novel pathogenic mechanisms and the identification of new biomarkers in osteoporosis or as potential drug-targets.

P17

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

Maria Venetikidou[1], Konstantina Balaska[2], Georgia-Persephoni Voulgaridou[1], Yiannis Karakasiliotis[3], Alexandra Giatromanolaki[2], Aglaia Pappa[1]

[1]Department of Molecular Biology and Genetics, School of Health Sciences, Democritus University of Thrace, Alexandroupolis, Greece
[2]Department of Pathology, University Hospital of Alexandroupolis, Democritus University of Thrace, Alexandroupolis, Greece
[3]Laboratory of Biology, Department of Medicine, School of Health Sciences, Democritus University of Thrace, Alexandroupolis, Greece

Lung cancer is among the most frequent malignancies worldwide, characterized by high mortality rate and is strongly related to tobacco smoking. The majority of reported cases concerns non-small cell lung cancer (NSCLC), being further subdivided in distinct subtypes. The second most common is squamous

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

P18

Differentiated ECM composition in 3D breast cancer cell spheroids

Konstantina Cheli[1], Georgios Baroutas[1], Zoi Piperigkou[1,2]*, Christos Koutsakis[1], Nikos K. Karamanos[1,2]

[1]Biochemistry, Biochemical Analysis & Matrix Pathobiology Research Group, Laboratory of Biochemistry, Department of Chemistry, University of Patras, Patras, Greece
[2]Foundation for Research and Technology-Hellas (FORTH)/ Institute of Chemical Engineering Sciences (ICE-HT), Patras 26110, Greece

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

P19

The *in vitro* anticancer activity of a σ2 agonist siramesine in pancreatic cancer

Fani Koutsougianni, Nikoleta Giovanovits, Dimitra Alexopoulou, Dimitris Magouliotis, Konstantinos Dimas

Department of Pharmacology, Faculty of Medicine, University of Thessaly, Larisa, Greece

Objective: Siramesine (SRM), is an agonist of σ2 receptors that is reported to show a promising antiproliferative and cytotoxic activity in tumor cells *in vitro* as well as *in vivo*. The aim of this study is the investigation of the *in vitro* activity of Siramesine both in the established human pancreatic cancer cell lines PANC-1 & BxPC3 and in an *ex vivo* pancreatic cancer cell population named Attached which was isolated in our laboratory directly by a patient derived xenograft.
Materials & Methods: We used the methods of SRB cytotoxicity test to determine the GI50, TGI, and LC50 of siramesine against pancreatic cancer cells, the clonogenic assay, and the wound healing assay to investigate the cytotoxic activity of siramesine, the inhibiting activity of SRM on the ability of single cells to make clones, and on the ability of the specific cells to migrate, respectively. Furthermore, through flow cytometry, we studied the effect of the compound on the cell cycle of pancreatic cancer cells to determine if the activity is cell cycle phase-specific.
Results: The data of this study, confirmed that siramesine has a strong antiproliferative and cytotoxic activity under the experimental conditions that have been tested. Moreover, siramesine was found to inhibit the ability of single cells to create colonies and to migrate in a time and dose-dependent manner. The flow cytometry data suggest that siramesine induces cell cycle arrest at the G0/1 phase of the cell cycle.
Conclusions: Siramesine, under the experimental conditions tested herein, exhibits strong anti-clonogenic and anti-migratory activity. Furthermore, we show that the activity of the compound is cell-cycle phase-specific against pancreatic cancer cells. These data are reported for the first time for siramesine. Further studies on the mechanism via which the compound exhibits these effects are ongoing.

P20

Pipeline development for the mutational analysis of beta-thalassemia patients

Sotiris Mavromatis, Eirini Veltsou, Marina Arbi, Zoi Lygerou

Molecular Genetics Unit, Medical School, University of Patras, Greece

Beta thalassemia is a hereditary blood disorder characterized by abnormal synthesis of hemoglobin beta chains, which can either be absent (β0) or reduced (β+). It is an autosomal recessive disease with variable phenotypes ranging from severe anemia (thalassemia major, intermedia) to clinically asymptomatic individuals (carrier state). Molecularly, β-thalassemia is very heterogeneous, with more than 200 disease-causing mutations having been identified until now. Most of them include single nucleotide substitutions, deletions, or insertions, while gross gene deletions are rare1. The incidence of β-thalassemia trait in the broader Mediterranean area is estimated to be 7-8%, considered to be high compared to the rest of the European Union2,3. Therefore, a reliable, cost-effective, and rapid scanning method for the beta globin gene point mutations, is necessary. The phenotype of beta-thalassemia can be modified by factors mapping outside the globin gene cluster, highlighting the need of scanning the patients' genome1. Here, we developed an assay for the mutational analysis of the beta globin gene for thalassemic patients, which could easily be adapted to a clinical laboratory. It includes the amplification of the β-globin gene via polymerase chain reaction (PCR), followed by Sanger sequencing analysis. The pipeline for sequencing analysis filters the variants based on the quality of the sequencing and the number of reads. The detected variants are annotated and called based on the ClinVar database,

however novel variants can be unveiled too. In the future, this pipeline could easily be adapted for analysis of multiple genomic regions, assisting to map possible mutations outside the beta globin locus.

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P21

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

Isidoros Axiotis[1,2], Efstathia Thymiakou[1,2], Katerina Karagkouni-Dalakoura[1,2] and Dimitris Kardassis[1,2]

[1]Laboratory of Biochemistry, Division of Basic Sciences, University of Crete Medical School, Heraklion, Greece
[2]Gene Regulation and Genomics group, Institute of Molecular Biology and Biotechnology, FORTH, Heraklion, Greece

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

P22

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

Georgios Kalampounias[1], Kalliopi Zafeiropoulou[1], Lydia Menounou[1], Apostolos Vlachos[1], Spyridon Alexis[2], Anargyros Symeonidis[2], and Panagiotis Katsoris[1]

[1]University of Patras, Department of Biology, Patras, Greece
[2]Hematology Division, Internal Medicine Department, University General Hospital of Patras, Patras, Greece

Carfilzomib (Kyprolis ®) is a second-generation proteasome inhibitor utilized to treat multiple myeloma in patients that have been previously treated with Bortezomib and disease progression is observed due to drug resistance. Carfilzomib exhibits more side effects than Bortezomib and for that reason is always used as a second-line medication even though it is believed to have the same target inside cells, targeting the β5 subunit of the 26S proteasome. By disrupting the β5 subunit chymotrypsin-like activity, the proteasome is deactivated, and the protein degradation pathways are inextricably disrupted causing the accumulation of damaged proteins and eventually the apoptotic death of the cell that is unable to maintain its homeostasis. Cancer cells are more susceptible to the deregulation of this system due to the higher biosynthetic rates they have compared to normal cells. In this study, Carfilzomib is administered to different groups of the DU-145 cancer cell line; cells resistant to Bortezomib, cells resistant to Carfilzomib, cells that have acquired resistance to Carfilzomib after continuous growth with Bortezomib, and naïve cells, and the main differences on cell viability, proliferation, and migration rates are recorded. Carfilzomib has fewer off-target effects being more specific and safer than Bortezomib but the development of cross-resistance to both substances is to be expected so the unveiling of causing mechanisms will allow the improvement of the current therapeutic approach and the design of more safe and effective treatments.

P23

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

Kalliopi Zafeiropoulou[1], Georgios Kalampounias[1], Spyridon Alexis[2], Daniel Anastasopoulos[1], Anargyros Symeonidis[2], and Panagiotis Katsoris[1]

[1]University of Patras, Department of Biology, Patras, Greece
[2]Hematology Division, Internal Medicine Department, University General Hospital of Patras, Patras, Greece

The proteasome inhibitor Bortezomib (Velcade®) is a drug administrated to treat multiple myeloma and has potential use as a treatment against prostate cancer and other types of malignancies. Although it has many positive aspects exhibiting low toxicity and few side effects, the main disadvantage is the appearance of resistant cell populations that manage to survive and are usually more aggressive requiring stronger medications. Bortezomib acts by specifically binding to the β5 subunit of the 26S proteasome and disrupts the protein degradation pathways leading to increased stress and eventually cell death by induction of apoptosis pathways. Cancer cells are more susceptible to proteasome disorganization due to the increased protein synthesis that leads to the accumulation of defective polypeptides. Within some weeks of administration phenomena of resistance are documented, mainly because of mutations at the PSMB5 gene, the gene coding for the β5 subunit, and the mutated clones have lower affinity for the drug as well as increased levels of 26S proteasome to stave off the effects of the drug. In this study, Bortezomib-resistant cell populations are created and subsequently, we try to uncover the phenotypic changes a resistant cell compiles. The main targets include survival, proliferation, migration, and apoptosis signaling pathways and response to oxidative stress caused by the drug, to understand better the differences between resistant and non-resistant cells on the prostate cancer cell lines DU-145. The differences between the two groups could act as therapeutic targets if a universal mechanism emerges.

P24

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

Georgios Kalampounias[1], Elena Anagnostopoulou[2], Chrysavgi Gardeli[2], Seraphim Papanikolaou[2], and Panagiotis Katsoris[1]

[1]Department of Biology, Division of Genetics, Cell and Development Biology, University of Patras, Greece
[2]Department of Food Science and Human Nutrition, Agricultural University of Athens, Greece

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

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P25

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

Georgios Kalampounias[1], Athina Varemmenou[1], Christos Aronis[1], Athanasios-Nasir Shaukat[2], Constantinos Stathopoulos[2], Dionysios Chartoumpekis[3], Marina Michalaki[3], and Panagiotis Katsoris[1]

[1]Department of Biology, University of Patras, 26504 Patras, Greece
[2]Department of Biochemistry, School of Medicine, University of Patras, 26504 Patras, Greece
[3]Endocrine Division of Medical School of Patras, University of Patras, 26504 Patras, Greece

Thyrotropin (TSH) is considered a mitogen for thyroid cells thus TSH suppression is required in the management of patients with Papillary Thyroid Carcinoma (PTC). However, recent data suggest that the molecular alterations implicated in thyroid tumorigenesis are mainly the activation of receptor tyrosine kinase (RTK) pathways, such as the mitogen-activated protein kinase (MAPK) and the phosphoinositide 3 kinase (PI3K), and the role of TSH is overlooked. If this is true, then a substantial number of PTC patients suffer from iatrogenic hyperthyroidism needlessly (via negative feedback inhibition of TSH secretion). To study this, the thyroid cell line Nthy-Ori 3-1 is utilized as a normal thyrocyte model and the cancer cell lines K1 and TPC-1 are used to monitor the effects of TSH on carcinoma cells. We used

recombinant human TSH (rh-TSH) and not bovine TSH (b-TSH) used in previous studies as it is expected to mimic better the conditions inside the human body. In this study, the hormone is administrated either alone or with the presence of insulin to monitor these synergistic effects. To multiply the effects of the hormone, cell clones (from the three cell lines) that stably overexpress the TSH receptor were prepared using a lentiviral vector/Tet operon system, and the increased expression levels were verified both at the RNA and protein levels. No significant changes in the tested parameters were detected disproving the role of TSH as a mitogenic agent without the synergistic function of other signaling molecules.

P26

Serglycin-mediated WISP-1 expression controls glioblastoma cell functions

Panagiotis Fountas, 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. Serglycin is a proteoglycan that can regulate the aggressive phenotype in several tumors, including GBM. Serglycin is secreted in the extracellular matrix and activate multiple oncogenic signaling pathways. WISP1 is a secreted cysteine rich growth factor that plays a vital role in embryonic stem cells controlling their functional properties. However, WISP1 also found to contribute to the progression of various tumors. WISP1 expression is upregulated in GBM compared to nonmalignant tissues, whereas its downregulation lead to reduced cell proliferation, migration, invasion and stemness phenotype of GBM cells. WISP1 is reported to signal through integrins and activate a plethora of signaling pathways involved in tumor cell behavior. The interaction of WISP1 with αvβ3 can manipulate crucial cellular functions, while its interaction with α6β1 can maintain the stemness capacity of glioma stem cells. Our laboratory has generated LN18 GBM cells with suppressed levels of serglycin (LN18shSRGN), which are characterized by reduced proliferation and migration rates, as well as diminished ability to form tumors *in vivo*. The expression levels of WISP1 are downregulated in LN18shSRGN cells. This is also associated with a significant reduction in mRNA and protein expression levels of various integrins. Using exogenously added recombinant WISP1, we found that LN18shSRGN increase their ability to proliferate, migrate and invade as well as to form colonies. Treatment with WISP-1 also increased the expression of β-catenin and proteolytic enzymes. The increased expression of WISP-1 is also associated with reduced overall survival in GBM. It is proposed that serglycin oncogenic properties are partially mediated through the regulation of WISP1 expression in GBM cells.

P27

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

Anastasios Christoforakos[1], Katerina-Marina Pilala[1], Marieta Xagorari[2], Antonios Marmarinos[2], Lydia Kossiva[3], Margarita Baka[4], Dimitrios Doganis[4], Marina Servitzoglou[4], Maria Tsolia[3], Andreas Scorilas[1], Dimitrios Gourgiotis[2], Margaritis Avgeris[1,2]*

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

[4]Department of Pediatric Oncology, "P. & A. Kyriakou" Children's Hospital, Athens, Greece.
Email: margaritis.avgeris@gmail.com; mavgeris@med.uoa.gr

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

P28

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

Aikaterini Diseri[1], Ioanna Maria Gkotinakou[1], Christina Befani[1], Martina Samiotaki[2], George Panayotou[2] and Panagiotis Liakos[1]*

[1]Laboratory of Biochemistry, Faculty of Medicine, University of Thessaly, Biopolis 41500, Larissa, Greece
[2]Institute of Bioinnovation, BSRC "Alexander Fleming," Vari 16672, Athens, Greece
*Email: pliakos@med.uth.gr

Hypoxia-inducible factors (HIFs) are the master transcription factors that regulate cellular responses to hypoxia. The HIF family contains HIF-1 and HIF-2 that function as heterodimers, with an oxygen-regulated α subunit and a stably expressed β subunit, also known as ARNT. HIF-2α is the less-studied isoform. Its expression is restricted to specific cell types and is involved in erythropoiesis, angiogenesis and metastasis. HIF-2α is controlled so by oxygen-dependent as by oxygen-independent mechanisms, such as posttranslational modifications and interaction with other proteins. Its so far known interactions have not been sufficiently studied. Thus, we have researched for new HIF-2α protein interactions. We have recently demonstrated that HIF-2α interacts with Reptin52 and this interaction impairs HIF-2 transcriptional activity (1).. In addition, we have distinguished a new protein that bounds to HIF-2α, which was identified by mass spectroscopy as Ataxin10. Ataxin10 is a protein that may function in cell survival, cytokinesis and differentiation.
In this study, to further investigate the involvement of Ataxin10 in the regulation of HIF-2α, the expression of Ataxin10 was suppressed in HeLa cells by small interfering RNA (siRNA)-mediated silencing and we observed by RT-PCR reduced mRNA expression levels of the HIF-2-specific target genes such as PAI-1, CyclinD1, Catalase and Superoxide Dismutase 2, suggesting inhibition of HIF-2 transcriptional activity. In corroboration, Flag-Ataxin10 overexpression experiments led to an increase in HIF-2 transcriptional

activity measured with Luciferase assay. Currently, we investigate the effect of HIF-2α interaction with Ataxin10 in cells adaptation under hypoxia.
Our findings highlight Ataxin10 as a novel protein that regulates HIF2 activity, and their crosstalk remains to be tested.

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P29

Dissecting the role of a putative DEAH-box RNA helicase 35 (DHX35)

Katerina Gentekaki, Eleni G. Kaliatsi, Constantinos Stathopoulos, Vassiliki Stamatopoulou*

Department of Biochemistry, School of Medicine, University of Patras, Greece

Helicases are ubiquitous enzymes present in all kingdoms of life and are essential in all aspects of nucleic acid metabolism. Helicases are mainly involved in unwinding specifically DNA, RNA or DNA-RNA hybrids and remodeling ribonucleoprotein complexes. Based on our previous work, we identified a putative ATP-dependent RNA helicase implicated in ribosomal RNA synthesis and processing, termed DEAH-box polypeptide 35 (DHX35). Interestingly, we detected a diverse expression profile of DHX35 not only in melanoma and lung cancer cell lines but also in samples derived from patients. This observation prompted us to further characterize DHX35 to unravel its biological function and mechanistic role in the malignancy onset and progress. Therefore, we first cloned and overexpressed DHX35 in an E. coli heterologous system. Recombinant DHX35 was then purified by size exclusion chromatography and was tested for substrates binding. Of note, DHX35 appears able to bind specific RNA molecules, and in particular snoRNAs (e.g., SNORD78 and SNORA73B) and precursor tRNAs as we showed by conducting Electrophoretic mobility shift assay. However, DHX35 could not bind any DNA substrate. Finally, we further achieved to knock out DHX35 in HeLa cell line by using CRISPR/Cas9 genome editing tool in an effort to thoroughly divulge the DHX35 biological role.

P30

The role of transcription factor TFAP2A in the hypoxic transcriptional control

Amalia Kanoura[1], Antonis Giakountis[2], Chrysa Filippopoulou[1], George Stamatakis[3], Martina Samiotaki[3], George Panayotou[3], George Simos[1] and Georgia Chachami[1] *

[1]Laboratory of Biochemistry, Faculty of Medicine, University of Thessaly, Biopolis 41500, Larissa, Greece
[2]Department of Biochemistry and Biotechnology, University of Thessaly, Larissa 41500, Greece
[3]Institute for Bioinnovation, Biomedical Sciences Research Center "Alexander Fleming", 16672, Vari, Attica, Greece
*Email: ghah@med.uth.gr

Cancer cells are exposed to hypoxic microenvironment due to their high proliferation rate and inadequate tumor vasculature. Hypoxia, via induction of HIFs, orchestrates reprogramming of gene expression that facilitates cellular adaptation. Sumoylation, covalent attachment of Small Ubiquitin related Modifier (SUMO) to proteins, has been lately implicated in the regulation of protein components of the hypoxic response. Using SUMO-immunoprecipitation in HeLa cells combined with quantitative proteomics we have identified several proteins, mainly transcription factors, with altered sumoylation status under

hypoxia1. One of the proteins with decreased sumoylation under hypoxia was TFAP2A, a transcription factor regulating a variety of cell processes including cell growth, differentiation and apoptosis. We have subsequently shown that TFAP2A physically interacts with HIF-1α and its sumoylation status affects HIF-1 transcriptional activity1. In order to characterize the interaction between TFAP2A and chromatin of hypoxia-inducible genes, we have performed ChIP-seq analysis using antibodies against TFAP2A or HIF-1α. We could show that TFAP2A binds to promoters of a set of known hypoxic (mostly glycolytic) genes together with HIF-1α. Moreover, silencing of TFAP2A inhibited their expression under hypoxia, suggesting that TFAP2A is a positive regulator of the hypoxic response. To further explore the involvement of TFAP2A in the transcriptional response to hypoxia, chimeric TFAP2A forms (wt, fully sumoylated, non-sumoylated) were overexpressed and their immunoprecipitates are being analyzed by mass spectroscopy. Our results so far suggest that TFAP2A may serve as a chromatin anchorage for HIF-1α and/or facilitate the recruitment of transcriptional machinery components. As both TFAP2A and HIFs have critical roles in oncogenesis, revealing the functional significance of their interaction can lead to the development of novel strategies for targeting and killing cancer cells in hypoxic tumors.

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P31

P1 tRNA^{Gly} ablation from *Staphylococcus aureus* deregulates iron homeostasis, tRNA pool and susceptibility to antibiotics

Adamantia Kouvela[1#], Nikoleta Giarimoglou[1#], Alexandros Maniatis[1], Jinwei Zhang[2], Constantinos Stathopoulos[1*], Vassiliki Stamatopoulou[1*]

[1]Department of Biochemistry, School of Medicine, University of Patras, Greece
[2]Laboratory of Molecular Biology, National Institute of Diabetes and Digestive and Kidney Diseases, 50 South Drive, Bethesda, MD 20892, USA
*Emails: cstath@med.upatras.gr; v.stam@upatras.gr
# Equal contribution

Besides the established role of tRNA in translation, it has been currently proven to possess additional non-canonical functions crucial for bacterial homeostasis and pathogenicity. Our previous work revealed the tRNA-mediated synchronization of translation and cell wall synthesis in the human pathogen *Staphylococcus aureus*. Specifically, five distinct tRNAGly isoacceptors exist in this pathogen, two of which are specialized to participate to protein translation and are called proteinogenic (P1, P2), while three are termed non-proteinogenic (NP1, NP2, New) and contribute to cell wall stabilization. Moreover, all tRNAsGly regulate the transcription of the glycyl-tRNA synthetase (GlyRS), an essential enzyme responsible for charging tRNAsGly, through binding to a structurally unique glyS T-box riboswitch. Interestingly, we showed that mainstream antibiotics bind to this special tRNAGly:glyS T-box complex and affect diversely the expression of GlyRS, highlighting a novel appealing molecular strategy against staphylococcal infections. Therefore, in order to unravel in-depth the role of each tRNAGly we knocked out the proteinogenic P1 tRNAGly by utilizing the CRISPR/Cas9 genome editing system. Although the edited strain grows similarly to the wild type, it appears more susceptible to antibiotics which prominent target is the cell wall. Interestingly, transcriptome analysis of the edited strain revealed differential expression of genes implicated to the iron homeostasis, which is a key process for bacterial infectivity and pathogenicity. Although, the non-proteinogenic tRNAsGly are upregulated, genes essential for the cell wall formation and the glyS T-box are downregulated. The purine metabolism and the redox status are also affected. Remarkably, variations of other tRNA species expression and an increase in the non-

proteinogenic tRNAGly halves are observed. Our results present the basis of a tRNA-mediated interconnection of distinct cellular processes that could be further exploited to fight species-specific infections.

P32

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

Nikoleta Pateraki[1], Evgenia Ntini[2]*

[1]Department of Biology, University of Crete, Heraklion, Crete, Greece
[2]Institute of Molecular Biology and Biotechnology of the Foundation for Research and Technology Hellas, Heraklion, Greece
* Corresponding author

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

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P33

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

Odysseas Panagiotis Tzortzatos[1], Dimitra K. Toubanaki[1], Paraskevi Zisimopoulou[2], Yannis Kotzamanis[3], Markos Kolygas[4], Fotini Athanassopoulou[4], Evdokia Karagouni[1]*

[1]Hellenic Pasteur Institute, Department of Microbiology, 11521, Athens, Greece
[2]Hellenic Pasteur Institute Department of Neurobiology, 11521, Athens, Greece
[3]Hellenic Centre for Marine Research, Biotechnology and Aquaculture, Institute of Marine Biology, 19013 Attiki, Greece
[4]University of Thessaly, Faculty of Veterinary Medicine, Ichthyology & Aquaculture 43100 Karditsa, Greece
*Email: ekaragouni@pasteur.gr

Fish infectious diseases are one of the main constraints of the aquaculture sector, representing a serious economic, social, and environmental challenge for the industry. The use of medicinal plants in aquaculture provides a sustainable way of protection using safe, eco-friendly compounds in a more cost effective way of treatment, compared to antibiotics and chemical compound currently used, due to their antimicrobial, immunostimulant, antioxidant, anti-stress, and growth-promoting properties1,2.
Aim of the present study was the assessment of *Artemisia arborescens* and *Thymus vulgaris* feed supplementation effects on sea bream (*Sparus aurata*) immune responses. Fish were divided in 5 groups based on feed composition: a) control group - commercial diet, b) group 0.25% *T. vulgaris*, c) group 0.50% *T. vulgaris*, d) group 0.25% A*. arborescens* and e) group 0.50% *A. arborescens*. After two months of experimental fish ad libitum feeding, the effect of diets on fish weight and length were measured. Immunological parameters (i.e., nitric oxide, lysozyme, total protein) were determined on fish serum and/or mucus. Spleen samples were subjected to qRT-PCR to evaluate expression levels of genes related to antioxidants (SOD1, GPx1), cytokines (Il10, TGFb1, Il-1b, TNFa), antibacterial peptide (Hepcidin) and heat shock protein (GRP75). Fish weight and length in most diet groups showed no significant differences. Serum nitric oxide (NO) levels were increased in the 0.50% *T. vulgaris* group, while in 0.25% *A. arborescens* group the concentration of NO almost doubled compared to control group. The genes expression levels increased in both diets with high extract concentration. In the present study, the suitability of *A. arborescens* and *T. vulgaris* as efficient food supplements for immune status improvement was investigated. The results indicated that *A. arborescens* and *T. vulgaris* could be used as dietary supplements since they appear to have considerable potential as natural immunostimulants.

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P34

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

Maria Anesti[1]*, Stefanos Kaplanis[2], Cédric Ghevaert[3], Domna Karagogeos[2], Ilias Kazanis[1]

[1]Laboratory of Developmental Biology, Division of Genetics Cell and Developmental Biology, Department of Biology, University of Patras, Greece
[2]Department of Basic Science, Faculty of Medicine, University of Crete and Institute of Molecular Biology & Biotechnology - FORTH, Heraklion, Greece
[3]Wellcome Trust – MRC Cambridge Stem Cell Institute & Department of Haematology, University of Cambridge, Cambridge, UK
*Email: anestimaria@gmail.com

In the postnatal mammalian brain Neural Stem and Progenitor Cells (NSPCs) are located within specialized microenvironments, called stem cell niches, one of which is the Subependymal Zone (SEZ) of the lateral walls of the lateral ventricles. Previous *in vivo* work has revealed that focal demyelination in the adjacent corpus callosum (CC) affects the SEZ, increasing the number of proliferating SEZ-resident NSPCs, an effect that is correlated with the specific aggregation of platelets in the niche vasculature (1).

In addition, using a coculture assay of platelets and NSPCs, it has been demonstrated that platelets affect the behaviour of the latter in differentiation and proliferation conditions. Here, we investigate further if these effects are due to direct cell-to-cell interactions, performing cocultures, as well as NSPC cultures in medium that has been enriched with platelet factors (conditioned medium). Preliminary results reveal that in the presence of high platelet densities the percentage of Sox2 immunopositive NSPCs increases in differentiation conditions, an effect that is not repeated when cultured in conditioned medium, reinforcing the role of cell-to-cell interactions between platelets and NSPCs. We also extend our *in vivo* analysis, performing experiments of focal demyelinating lesion on the CC in animals with reduced numbers of platelets, using two different procedures: transient chemical platelet depletion, as well as thrombocytopenic Nbeal2 knockout mice that have thrombocytopenia and platelets that are lacking functional α-granules (functional thrombocytopenia).

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P35

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

Katerina Apostolidi[1,2], Maria Markou[1], Sofia Bellou[1,4], Theodore Fotsis[1,3], Carol Murphy[1]*, Eleni Bagli[1]*

[1]Foundation of Research and Technology-Hellas, Biomedical Research Institute, University Campus, 45110 Ioannina, Greece
[2]Department of Biological Applications and Technology, University of Ioannina, 45110 Ioannina, Greece
[3]Laboratory of Biological Chemistry, Medical School, University of Ioannina, 45110 Ioannina, Greece
[4]Confocal Laser Scanning Microscopy Unit, Network of Research Supporting Laboratories, University of Ioannina, Ioannina, 45110, Greece

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

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

Dimitrios Dimitrakopoulos[1], Christina Dimitriou[1], Freya McClenahan[2], Robin J. M. Franklin[2,3], Ilias Kazanis[1]

[1]Laboratory of Developmental Biology, Department of Biology, University of Patras, Greece
[2]Wellcome Trust – MRC Cambridge Stem Cell Institute, University of Cambridge, United Kingdom
[3]Altos Labs, Cambridge Institute of Science, Granta Park, Cambridge CB21 6GP, United Kingdom

Postnatal brain neural stem cells (pbNSCs) remain active in specialized niches, where they generate new neurons and glia. One such niche is the subependymal zone (SEZ/ also called the subventricular zone) that is located across the lateral walls of the lateral ventricles adjacent to ependymal cells. Oligodendrocyte progenitor cells (OPCs) are abundantly distributed throughout the central nervous system, constituting a pool of proliferative progenitor cells that can generate oligodendrocytes. Both pbNSCs and OPCs exhibit self-renewal potential and quiescence. Due to their location, the isolation of these cells requires the sacrifice of experimental animals. Here, we describe in detail “brain milking”, a method for the isolation pbNSCs and OPCs from live animals. The protocol has been developed in rats and includes two major steps. First, pbNSCs of the SVZ are ‘‘released’’ from the tissue via the stereotaxic intracerebroventricular injection of a ‘‘release cocktail’’ that contains a) neuraminidase, a toxin that induces ventricular wall denudation by targeting specifically ependymal cells. b) an integin-b1-blocking

antibody, and c) fibroblast growth factor 2. At a second ‘‘collection’’ step, liquid biopsies of cerebrospinal fluid are performed from the cisterna magna, in anesthetized rats without the need of an incision. Several liquid biopsies can be taken from the same animal on the same and at consecutive days, allowing the performance of longitudinal experiments. By changing the injection co-ordinates, the isolation of different pools of stem/progenitor cells can be achieved.

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

Panagiota Giannakopoulou[1], Korina Atsopardi[1,2,3]*, Dimitrios E. Providas[3], Demetrios Kouretas[4], Konstantinos Poulas[3], Marigoula Margarity[2], Ilias Kazanis[1]

[1]Laboratory of Developmental Biology, Department of Biology, University of Patras, Greece
[2]Laboratory of Human and Animal Physiology, Department of Biology, University of Patras, Greece
[3]Laboratory of Molecular Biology and Immunology, Department of Pharmacy, University of Patras, Greece
[4]Department of Biochemistry and Biotechnology, University of Thessaly, Larissa, Greece

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

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

Theodosia Androutsopoulou[1,2]*, Georgios Leventakos[1], Ioannis Tsounios[1], George Mitsainas[2], Ilias Kazanis[1]

[1]Laboratory of Developmental Biology, Department of Biology, University of Patras, Greece
[2]Laboratory of Animal Biology, Department of Biology, University of Patras, Greece

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

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E2 conjugating enzyme selectivity and enzymatic activity of E3 ligase Arkadia

Georgia N. Delegkou[1], Tamara Toro[1], Maria Birkou[1], Konstantinos D. Marousis1, Vasso Episkopou[2], Georgios A. Spyroulias[1]*

[1]Department of Pharmacy, University of Patras, Greece
[2]Department of Brain Sciences, Imperial College, London, UK
*Email: G.A.Spyroulias@upatras.gr

Ubiquitination is a fundamental post-translational modification that regulates a host of cellular functions, mainly by mediating the selective proteasomal degradation of regulatory proteins. The process is catalyzed by the concerted action of activating (E1), conjugating (E2) and ligating (E3) enzymes and its deregulation has been implicated as a causative factor in various diseases, including cancer. Arkadia/RNF111 is a RING E3 ubiquitin ligase that enhances the transforming growth factor-β (TGF-β) signaling responses, through ubiquitin-dependent degradation of negative regulators of the pathway i.e., the inhibitory SMAD7, transcriptional co-repressor SKI and its close homologue SNON (SKIL)1. Arkadia is also classified as a SUMO-targeted ubiquitin ligase (STUbL) implicated in the DNA damage response pathway. Specifically, Arkadia interacts with heterodimer UbcH13/MMS2 E2 complex to promote the formation of K63-linked ubiquitin chains to SUMOylated proteins e.g., Xeroderma Pigmentosum group C (XPC)2 and Promyelocytic Leukaemia protein (PML)3. Despite the pivotal role of Arkadia in many pathways, little is known about the features that modulate its enzymatic function. We have previously shown that, unlike most other E3 ligases, RING domain of Arkadia is not fully active and requires external elements to activate an E2~Ub complex4. This study has the potential to provide understanding on how Arkadia recognizes its physiological E2 partners and whether E2-Arkadia interaction leads to a productive complex. The interaction of Arkadia with the E2 partners UbcH5B, UbcH13, as well as UbcH7, is studied through NMR Spectroscopy. In this context we demonstrate that the interfaces of Arkadia-UbcH5B and Arkadia-UbcH13 complexes remain constant in both cases. In contrast, Arkadia-UbcH7 complex displays a relatively limited interaction surface in agreement with its inability to perform ubiquitination. Furthermore, we revealed that the synergistic action of Arkadia with different E2s, catalyzes either mono- or poly-ubiquitin chain formation, that can lead to distinct biological outcomes.

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Structural determination of the Phe964Ala Arkadia mutant via NMR Spectroscopy

Apostolos N. Koutsodimas[1], Georgia N. Delegkou[1], Maria Birkou[1], Vasso Episkopou[2], Georgios A. Spyroulias[1]

[1]Department of Pharmacy, University of Patras, Greece
[2]Department of Brain Sciences, Imperial College, London, UK
Email: G.A.Spyroulias@upatras.gr

Ubiquitination is a post-translational modification responsible for a variety of cellular functions including DNA repair, apoptosis and protein degradation. Initially, ubiquitin is activated by the E1 enzyme in an ATP-depended manner which results in the formation of a thioester bond between the glycine-76 of ubiquitin and a cysteine of the E1. Afterwards, the activated ubiquitin is transferred to the catalytic cysteine of the E2 conjugating enzyme. In the final step, the E3 ligase facilitates the transmission of ubiquitin to the substrate. The target protein may undergo several types of ubiquitination each of which results in diverse outcomes1.
Arkadia is an E3 ubiquitin ligase that positively regulates the transforming growth factor-β (TGF-β) pathway by promoting ubiquitin-dependent degradation of the negative regulators Smad7, c-Ski and SnoN. Arkadia possesses a RING-H2 domain at its C-terminus, which exhibits a canonical RING ββα topology consistent to other RING domains. The 3-turn a-helix and the two zinc-binding loops of Arkadia RING domain play a crucial role in the interaction with the E2 enzymes2. We have previously demonstrated that mutations in conserved residues of the RING domain result in structural alterations of the domain and therefore its interaction with the E2 enzyme3. In this study we investigate the pivotal role of Phe964 of Arkadia, a sequentially and structurally conserved residue in many RING domains, via NMR spectroscopy. This residue is located at the hydrophobic core of the protein and is considered a "linker" between the second and third binding motif. Phe964Arg mutant led the protein to an unfolded state, contrary to Phe964Ala mutant that was well-folded. Phe964Ala mutant displayed differences in the signal dispersion compared with the wild type one and exhibited no interaction with E2 UbcH5b. In order to estimate the impact of this mutation to the RING domain, structural, dynamical and functional characterization was conducted.

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Structural and functional studies of viral methyltransferase of Hepatitis E Virus

Aikaterini C. Angelopoulou[1], Maria D. Politi[1], Konstantina Kaperoni[1], Bruno Coutard[2], Georgios A. Spyroulias[1]*

[1]Department of Pharmacy, University of Patras, Patra, Greece
[2]AFMB, UMR 7257 CNRS/ University of Merseille, Merseille CEDEX 9, France
*Email: G.A.Spyroulias@upatras.gr

Hepatitis E virus (HEV) is identified as the most common cause of acute viral hepatitis worldwide. HEV infection is estimated to cause 70,000 deaths from acute liver failure. There are seven genotypes (HEV1-HEV7), yet only the first four genotypes are responsible for human infection. According to the statistical incidence data, 20 million cases of HEV infection occur annually, while approximately 3.3 million result in symptomatic infections. Transmission occurs mainly through the fecal-oral route, but also through contaminated blood transfusions, transplants, and from mother to embryo [1]. HEV belongs to the Hepeviridae family and its genome is a 7.2Kb ss(+) RNA. It consists of a 5' untranslated region (5'-UTR), 3 open reading frames (ORF's) and a 3' untranslated region(3'-UTR) which ends in a polyadenine tail [2]. Each ORF has a crucial role for a successful viral transmission and human infection. Our study attempts to elucidate the physicochemical and structural properties of two components of the ORF-1, which are necessary for RNA replication. These functional domains are the methyltransferase (vMT) and the macro domain (vMD). HEV vMT catalyzes the transfer of the methyl group from S-adenosylmethionine to GTP, resulting in m7GTP, a procedure known as mRNA capping. mRNA capping is essential for virus life cycle and the multiplication process [3]. Additionally, vMD serves not only a central role in a variety of cellular activities, including de-MARylation and de-PARylation, but also may be associated with persistent infection through the interaction with MT [4]. In the present study, we describe the cloning, expression, purification of vMT protein of HEV and the direct interference with the de-Marylation activity of vMD. Specifically, a variety of experiment conditions were tested for the expression of different recombinant polypeptides of vMT, to find the appropriate polypeptide for the structural and the protein-protein interaction studies through NMR spectroscopy.

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Structural studies of the SARS Unique Domain (SUD) of SARS-CoV-2 with its intracellular partner, the human protein RCHY1, through biophysical studies

Eftychia Matakouli, Nikolaos K. Fourkiotis, Aikaterini C. Tsika, Aikaterini I. Argyriou, Angelo Gallo, Georgios A. Spyroulias*

Department of Pharmacy, University of Patras, Greece

Severe acute respiratory syndrome coronavirus 2 (SARS CoV-2) is a highly pathogenic virus, cause of the worldwide pandemic COVID-19. Its positive sense, single stranded RNA encodes, among others, 16 non-structural proteins (nsPs), which form a replication–transcription complex (RTC) vital for the virus's proliferation inside the host.1 NsP3, the largest one, possesses multiple domains, including the SARS Unique Domains (SUDs). The N-terminal, middle, and C-terminal domains—abbreviated SUD-N, SUD-M, and SUD-C—are its three subdomains. The last domain adopts a frataxin-like fold, while the first two, a macro-like fold.2 Considering that SUDs of SARS CoV and SARS CoV-2 share a significant degree of amino acid sequence identity, it is assumed, that they will also share similar functions, in terms of participation in viral replication and interactions with the host's proteins. These include the ability, of SUD-N and -M domains, to bind guanine-rich, non-canonical nucleic acid structures known as G-quadruplexes and the regulation of the activity of the E3 ligase ring-finger and CHY zinc-finger domain-containing 1 protein (RCHY1).
RCHY1's most crucial regulator is the antiviral protein p53, while RCHY1 acts as a negative regulator of this factor, especially its transcriptionally active form. Therefore, increased levels of p53 lead to its poly-ubiquitination and, consequently, to its proteolytic degradation. Besides, interaction between RCHY1 and the SUDs of SARS-CoV stabilize the E3 ligase, which induces further the poly-ubiquitination of endogenous p53. The SUD domains bind to RCHY1 at a region between residues 95 and 144, while both N and M domains are required for the interaction.3 Nevertheless, the interaction between the SUDs of SARS CoV-2 and RCHY1 has not been clarified yet and it needs further investigation. Herein we use NMR to monitor titration experiments and to clarify the elucidation of the interaction between SUD-NM and RCHY1.

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Human transcription factor LRF regulates lncRNA and miRNA species' expression

Katerina Athanasopoulou[1], Vasiliki Chondrou[1], Panagiotis Xiropotamos[2], Georgios Psarias[1], Yiannis Vasilopoulos[2], George Georgakilas[2], Argyro Sgourou[1]*

[1]Biology Laboratory, School of Science and Technology, Hellenic Open University Patras, Greece
[2]Laboratory of Genetics, Section of Genetics, Cell Biology and Development, Department of Biology, University of Patras, Greece, *e-mail: sgourou@eap.gr

Human LRF (Leukaemia/lymphoma related), encoded from the ZBTB7A gene is a key epigenetic player in haemoglobin switching from fetal to adult stage. Apart from γ-globin genes inhibition co-directed with the master repressor BCL11A and BGLT3-lncRNA enhancement to promote open chromatin configuration, LFR shows broad binding preferences across genome, mainly at CG-rich and CpG island (CGI) containing promoters. We extended our research to genomic loci with LRF binding sites, driving the expression of lncRNAs and miRNAs to reveal potential LRF regulatory properties on non-coding RNA species' expression. Genomic loci of preference were retrieved from ChIP-seq analysis, previously conducted I.
Human untransfected K562 (erythroleukemia) cells and transgenic K562 clones, transfected with episomes overexpressing LFR (LRF-OE), were subjected to analysis. Genomic DNA extraction and bisulfite treatment followed by pyrosequencing uncovered methylation profiles of CGIs located at 5' prime ends of genes encoding lncRNAs. Total RNA was extracted from both untransfected and LRF-OE clones and cDNAs were tested with qPCR for selected lncRNA expression levels. Among lncRNAs, 10 with either unmethylated or not flanked by CGI showed half and less than half expression in LRF-OE clones, 3 with almost 100% methylated CGI were not expressed, while other 3 with CGI methylation between 30-80% had undetectable expression differences. 3 of the lncRNA species were outliers, which do not fall into any of the above categories. Furthermore, cDNA libraries were constructed with advanced methodology and sequenced on an Illumina i-seq 100 platform to perform both qualitative and quantitative detection of mature miRNAs. NGS results uncovered differentially expressed miRNAs between untransfected and LRF-OE clones, implicated in essential cell functions. Results of this study highlight the impact of LRF in non-coding RNAs expression regulation, mainly as a repressor. LRF exerts its indirect regulation in epigenetic events through down-regulation of non-coding RNAs and/or promoting methylation alteration in CGIs.

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P44

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

Markella V. Stogiannoudi[1], Elisavet M. Andronidou[1], Panagiota I. Kontou[2], Pantelis G. Bagos[1], Georgia G. Braliou[1]*

[1]Department of Computer Science and Biomedical Informatics, University of Thessaly, Lamia, Greece
[2]Department of Mathematics, University of Thessaly, Lamia, Greece

Iron is an essential nutrient for both humans and pathogenic microorganisms that infect them. It is an indispensable metal that serves as a cofactor for many enzymes regulating vital cellular functions.

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

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

Marina Giannakara, Vassiliki Lila Koumandou*

Department of Biotechnology, Agricultural University of Athens, Greece

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

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P46

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

Ourania Preza[1], Nibal Badra Fajardo[1], Sébastien Tosi[3], Julien Colombelli[3], Stavros Taraviras[2], Zoi Lygerou[1]*

[1]Laboratory of General Biology, School of Medicine, University of Patras, Greece
[2]Laboratory of Physiology, School of Medicine, University of Patras, Greece
[3]Advanced Digital Microscopy Core Facility, Institute of Research in Biomedicine, Spain

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

P47

Propolis: a biotechnological approach screening for antibacterial activity

Stavroula Mamoucha[1], Vasileios Liapis[2], Anastasia Prompona[1]

[1]Institute of Biosciences and Applications, National Centre for Scientific Research DEMOKRITOS, Ag. Paraskevi, Attiki
[2]Microbiological Laboratory of the M.T.S. Hospital Foundation. Monis Petrakis 10-12

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

The pharmaceutical and cosmetic areas could benefit from this product, which could result in better disease treatment and the improvement of cosmetics with esthetic aspects.

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

P48

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

Eleni Kalafati[1], Ekati Drakopoulou[1], Tina Bagratuni[2], Evangelos Terpos[2], Eugenia Tsempera[1], Maria K. Angelopoulou[3], François L. Cosset[4], Els Verhoeyen[4,5], Kostas Konstantopoulos[3], Eleni Papanikolaou[6], Nicholas P. Anagnou[1,6]*

[1]Laboratory of Cell and Gene Therapy, Biomedical Research Foundation of the Academy of Athens, Greece
[2]Plasma Cell Dyscrasia Unit, Department of Clinical Therapeutics, School of Medicine National and Kapodistrian University of Athens, Alexandra Hospital, Athens, Greece
[3]Department of Hematology and Bone Marrow Transplantation Unit, University of Athens School of Medicine, Athens, Greece
[4]International Centre for Infectiology Research, INSERM, U-1111, Université de Lyon, France
[5]INSERM, U-1065, Université Côte d'Azur, Nice, France
[6]Laboatory of Biology, School of Medicine, National and Kapodistrian University of Athens, Greece

Multiple myeloma (MM) represents a malignant plasma cell disorder characterized by severe clinical manifestations and complications. In the context of developing novel therapeutic approaches for MM, we investigated the efficacy of an IFNβ-expressing lentiviral vector, pseudotyped with the measles virus H and F glycoproteins (IFNβ/HF) in myeloma cell lines and in MM patients′ CD138+cells.
Cytotoxicity was evaluated using CCK-8. Apoptosis and cell cycle were estimated by AnnexinV/7-AAD and PI staining, respectively. Expression of apoptotic genes was determined by qPCR and a Human Apoptosis Antibody Array. IFNs secretion and its paracrine action were determined by ELISA and Transwell® co-culture. Autophagy gene expression was assessed employing qPCR and confocal microscopy. Neutralizing antibody activity was determined by flow cytometry utilizing GFP/HF.
IFNβ/HF exhibited a mean transduction efficiency of 29.5% (H929) and 23.1% (JJN3), with mean VCN/cell of 1.6 and 1.2, respectively. IFNβ/HF-transduction (MOI=1), led to a dramatic reduction of cell survival followed by a marked increase of apoptosis: 90.3% ($p \leq 0.001$) in H929, 74.1% ($p \leq 0.001$) in JJN3, 91.82% ($p = 0.004$) in U266, and 92.59 % ($p = 0.002$) in RPMI-8226 cells. Notably, the increase of Caspases 3 and 9 highlights the crucial role of the intrinsic apoptotic pathway in the IFNβ-induced apoptosis, whereas a decrease of autophagy gene expression (e.g. Beclin-1, Atg4b, Atg5) was detected. After Transwell® co-culture, IFNβ secreted by the transduced cells, was capable of significantly decreasing the viability of untransduced cells. Furthermore, IFNβ/HF efficiently transduced primary cells, increasing IFNβ and IFNγ secretion and decreasing cell survival, with a 51.8% increase of apoptosis ($p = 0.01$) and induction of cell cycle arrest. Since the antibody titer against measles virus is low, IFNβ/HF can overcome the immunological barrier using a higher MOI.
These data document that IFNβ/HF represents a promising therapeutic candidate for selective oncolytic action for MM.

P49

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

Anastasios-Konstantinos Sakellaridis[1], Ioannis Stathas[1], Natalia Tsouggou[1], Maria-Chrysanthi Kafentzi[1], Dimitrios Pavlidis[1], Nikos Papandreou[2], Vassiliki Iconomidou[2], John Kapolos[1], Marina Papadelli[1], Konstantinos Papadimitriou[3]*

[1]University of the Peloponnese, School of Agriculture and Foods, Department of Food Science and Technology, Kalamata, Greece
[2]Section of Cell Biology and Biophysics, Department of Biology, National and Kapodistrian University of Athens, Greece
[3]Agricultural University of Athens, Department of Food Science & Human Nutrition, Laboratory of Food Quality Control and Hygiene, Athens, Greece

In recent years, bioactive peptides present in foods and their potential beneficial effects for human health have been under rigorous investigation. Bioactive peptides are released during the fermentation process from the proteins of raw materials (e.g. milk and must) as a result of the proteolytic activity of the fermenting microbiome which is mainly comprised of lactic acid bacteria (LAB) and yeasts.The aim of our study was to collect the publicly-available peptidomics datasets from different wines and yogurt samples and re-evaluate in silico their physicochemical properties and functionalities using state-of-the-art bioinformatic tools.The majority of the peptides found in yogurt derived from the hydrolysis of β-casein, while others from αS1-, αS2-, and κ-casein. Very few peptides derived from the hydrolysis of whey proteins (β-LG and α-LA). Peptides in wine mainly come from the partial conversion of either naturally occurring proteins (i.e., the proteomes of Vitis vinifera and Saccharomyces cerevisiae) or from exogenous proteinsadded for technological reasons. For the physicochemical properties of the peptides, various parameters were calculated, including aliphatic index, and grand average of hydropathicity (GRAVY), using software tools such as ProtParam. For the functional characterization of the peptides, we used the following databases: FermFoodDB, DFBP, BioPepDB, BioPep-UWM and MBPDB. Some of the aforementioned databases, such as EROP-Moscow allowed us to correlate the peptides with the flavor of foods (sour, umami or bitter). The bioactive peptides were found to exhibit diverse activities, such as antioxidant, antihypertensive, antimicrobial, anti-inflammatory, immunomodulatory and opioid. Interestingly, some reported peptides present more than one functions. More studies need to be performed to further validate the bioactivity of these peptides so that they find application in the food industry.

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P50

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

Eleni Panagiotidou[1,2], Anna Gioran[1] and Niki Chondrogianni[1]

[1]Institute of Chemical Biology, National Hellenic Research Foundation, Athens, Greece
[2]Department of Biochemistry and Biotechnology, University of Thessaly, Larissa, Greece

The proteasome is one of the main proteolytic complexes of the cell, responsible for at least 80% of the protein degradation including the rapid degradation of misfolded and regulatory proteins. It is part of a highly conserved network, namely the Proteostasis Network (PN), that is responsible for the cellular protein homeostasis (proteostasis). The PN controls the quality of proteins from synthesis to folding and degradation and has been shown to become deregulated during the progression of aging and age-related

diseases, including proteinopathies. Given that the PN reinforcement has been proposed to be a promising approach in the design of preventive and/or therapeutic interventions against proteinopathies, it is critical to elucidate the regulation of the different PN components. Since different tissues are not equally affected under adverse conditions, tissue-communication through cell non-autonomous signaling pathways seems to be crucial for the proteome organismal integrity. Various players of the PN have been shown to be subjected to cell non-autonomous regulation through communication among tissues, with the exception of the proteasome where nothing has appeared so far. In this study, using *C. elegans* as a model, we investigated for the first time, whether the proteasome and its function may be regulated in a cell non-autonomous manner and the effects of such type of regulation upon proteotoxic stress. We reveal that proteasome activation in the neurons of the nematode can affect proteostasis in the muscle tissue. We also reveal that this communication requires the participation of a specific type of vesicles found in the synaptic transmission. Finally, we also show that this cell non-autonomous regulation is able to alleviate the proteotoxic phenotype in *C. elegans* models of Alzheimer's disease. In total, our results can provide important information for future targeted interventions.

P51

Increased concentration of IgG antibodies against egg-albumin in serum and cerebrospinal fluid of patients with Alzheimer's disease

Dionysia Amanatidou[1], Maria Myriouni[1], Magdalini Tsolaki[2], Anna Anastasiou[2], Athanasia Papageorgiou[2], Phaedra Eleftheriou[1]

[1]International Hellenic University, Thessaloniki, Greece
[2]Aristotle University of Thessaloniki, Thessaloniki, Greece

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

P52

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

Dionysia Amanatidou[1], Anthi Petrou[2], Athina Geronikaki [2], Phaedra Eleftheriou[1]*

[1]Department of Biomedical Sciences, School of Health, International Hellenic University, Thessaloniki, Greece
[2]Department of Medicinal Chemistry, School of Pharmacy, Aristotle University of Thessaloniki, Greece.

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

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P53

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

Athanasios A. Panagiotopoulos[1], Konstantina Kalyvianaki[1], Evangelia Konstantinou[1], George Notas[1], Stergios A. Pirintsos[2], Elias Castanas[1]*, Marilena Kampa[1]*

[1]Laboratory of Experimental Endocrinology, University of Crete, School of Medicine, Heraklion, Greece
[2]Department of Biology, School of Science and Technology, University of Crete, Heraklion, Greece
*Emails: kampam@uoc.gr, castanas@uoc.gr

Prostate cancer is known as hormone-sensitive, androgen dependent tumor and the second leading cause of cancer death in men. It is clear that androgens and androgen receptor signaling are crucial for prostate cancer growth and have been exploited therapeutically. However, hormone resistant prostate cancer is an unsolved problem with limited therapeutic choices. The action of androgens is mediated mainly through intracellular androgen receptors, which belong to the nuclear family of receptors. These receptors are transcription factors that determine key cell processes. A recent study by our team identified an alternative androgen receptor on the membrane of prostate cancer cells, OXER1 (5-oxo-6E, 8Z, 11Z, 14Z-eicosatetraenoic acid receptor). Intrestingly, androgens via OXER1 inhibit cancer cell growth and migration. The aim of this research was to identify new molecules that will bind to the membrane receptor of androgens, OXER1 and will have antagonistic effects such as testosterone. To achieve this, we focused on natural products which there were data that may have a pharmacological

effect and a therapeutic benefit in prostate cancer. Initially we performed in silico studies starting with the modeling of the interaction of OXER1 receptor with testosterone and 5-oxo-ETE. Due to the large number of natural products studied, an algorithm was designed and developed, allowing the fast and accurate classification of the examined chemical molecules. Next, using the advanced bioinformatics tool, OXER1 specific antagonists were identified. *In vitro* verification of the antagonistic properties of the selected compounds was performed in different cellular activities. The identified natural compounds, through bioinformatics methods, were tested in a number of cellular activities, related to the Gα and Gβγ activities of OXER1, such as cAMP, actin polymerization and their effect on calcium ion flow. In conclusion, the achievement of present work is the identification of compounds as specific antagonists of OXER1 via Gα and Gβγ activities. All these support testosterone actions at the membrane level, via OXER1, and provide new tools and agents for possible novel therapeutic approaches in cancer.

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P54

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

Vasileios Xanthis, Georgia-Persephoni Voulgaridou, Vasileios Theologidis, Eleni Papagiannaki, Ilias Tsochantaridis, Florentia Krasa, Vasiliki E. Fadouloglou*, and Aglaia Pappa*

Department of Molecular Biology & Genetics, Democritus University of Thrace, 68100 Alexandroupolis, Greece

Aldehyde dehydrogenase 3A1 (ALDH3A1) is a multifunctional antioxidant enzyme, which main activity is to oxidize medium chain aldehydes to their corresponding carboxylic acids. It is also implicated in various homeostatic mechanisms, such as cell proliferation, cell cycle regulation and DNA damage response. ALDH3A1 has been characterized as a potential cancer stem cell (CSC) marker in various types of solid tumor malignancies, such as melanoma, prostate, gastric and lung cancer. Additionally, ALDH3A1 is associated with chemo/radiotherapy resistance through its ability to oxidize toxic aldehydes. In the present study, we used a random 12-mer phage peptide display library and isolated four ALDH3A1-interacting peptides, one of which (P1) appeared to bind most efficiently to ALDH3A1. Bioinformatic analysis indicated two possible P1 binding sites on the protein surface indicating biomedical potential; Site 1 which is located near the substrate binding site, implying a specific binding to ALDH3A1, and Site 2 which is overlapping with the generally conserved cofactor binding region, suggesting binding to various isoforms of ALDH enzymes' family. Moreover, we created, through BLASTp search, a list of proteins similar to P1 sequence in order to find interacting partners. Protein Kinase C Binding Protein 1 and

General Transcription Factor II-I are amongst the likely candidates due to their cellular localization and biological function. Finally, we assessed the potential ALDH3A1 inhibitory potential of the P1 peptide and demonstrated that P1 significantly inhibits recombinant human ALDH3A1's enzymatic activity against benzaldehyde. In conclusion, this study identifies a novel peptide with potential use as an ALDH3A1 inhibitor and further suggests protein candidates to be explored as possible ALDH3A1-interacting partners in future studies.

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P55

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

Angeliki Kanellaki[1], Maria-Elpida Christopoulou[1,2], Stavros Meropoulis[3], Christos Aggelopoulos[3], Spyros S. Skandalis[1]*

[1]Biochemistry, Biochemical Analysis & Matrix Pathobiology Res. Group, Laboratory of Biochemistry, Department of Chemistry, University of Patras, Patras, Greece
[2]Department of Pneumology, Medical Center-University of Freiburg, Faculty of Medicine-University of Freiburg, Freiburg, Germany
[3]Laboratory of Cold Plasma and Advanced Techniques for Improving Environmental Systems, Institute of Chemical Engineering Sciences, Foundation for Research and Technology Hellas (FORTH/ICE-HT), Patras, Greece
*skandalis@upatras.gr

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

P56

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

Panagiota Karamitsou[1], Varvara-Christina Siagka[1], Maria-Elpida Christopoulou[1,2], Alexios Aletras[1], Spyros S. Skandalis[1]*
[1]Biochemistry, Biochemical Analysis & Matrix Pathobiology Res. Group, Laboratory of Biochemistry, Department of Chemistry, University of Patras, Patras, Greece
[2]Department of Pneumology, Medical Center-University of Freiburg, Faculty of Medicine-University of Freiburg, Freiburg, Germany
*skandalis@upatras.gr

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

P57

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

Anastasios Tsoungos[1], Dimitrios Pavlidis[1], Konstantinos Panousopoulos[1], Violeta Pemaj[1], Ioanna Theodoropoulou[1], Marina Papadelli[1], John Kapolos[1], Konstantinos Papadimitriou[1,2]*
[1]University of the Peloponnese, School of Agriculture and Foods, Department of Food Science and Technology, Antikalamos 24100, Kalamata, Greece
[2]Agricultural University of Athens, Department of Food Science & Human Nutrition, Laboratory of Food Quality Control and Hygiene, Iera Odos 75, 11855, Greece
*Email: kpapadimitriou@aua.gr

Even though the evolution of the microbiome of table olives during fermentation process has long been investigated, there is little, or no information regarding the microbial diversity at retail. Thus, the aim of the study was to assess different table olive cultivars for serving as a pool of new starters or adjunct cultures for the production of Greek-style olives. In total, nine olive samples from cv Kalamata, cv Konservolia, and Halkidiki were collected from large supermarkets. Respectively, commercially produced brines were analysed microbiologically (Lactic acid bacteria, enterococci leuconostocs, yeasts) and physicochemically (pH, acidity, %NaCl). Then, total DNA was extracted and subjected to Next Generation Sequencing for the exploration of bacteria and yeasts and molds community. The 16S and ITS rDNA amplicon sequencing analysis allowed the identification of bacterial populations at the genus level. Although differences were observed between the microbial abundance of the samples, the predominant genera were lactic acid bacteria and/or yeasts. Furthermore, the microbiota of the samples was identified

at the species level by shotgun metagenomics. The microbial contribution to the technological output of the final product was investigated in cells harvested from simulated olive broth. The results were comparable to the cultivable community identified by MALDI-ToF/ToF. The findings of the study showed that the commercially available table olives could contribute to the collection of microbial isolates with promising properties. In all cases, results should be validated with controlled fermentations.

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P58

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

Zacharias Faidon Brotzakis

[1]University of Cambridge, Cambridge, UK
[2]BSRC Al. Fleming, Athens, Greece

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

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