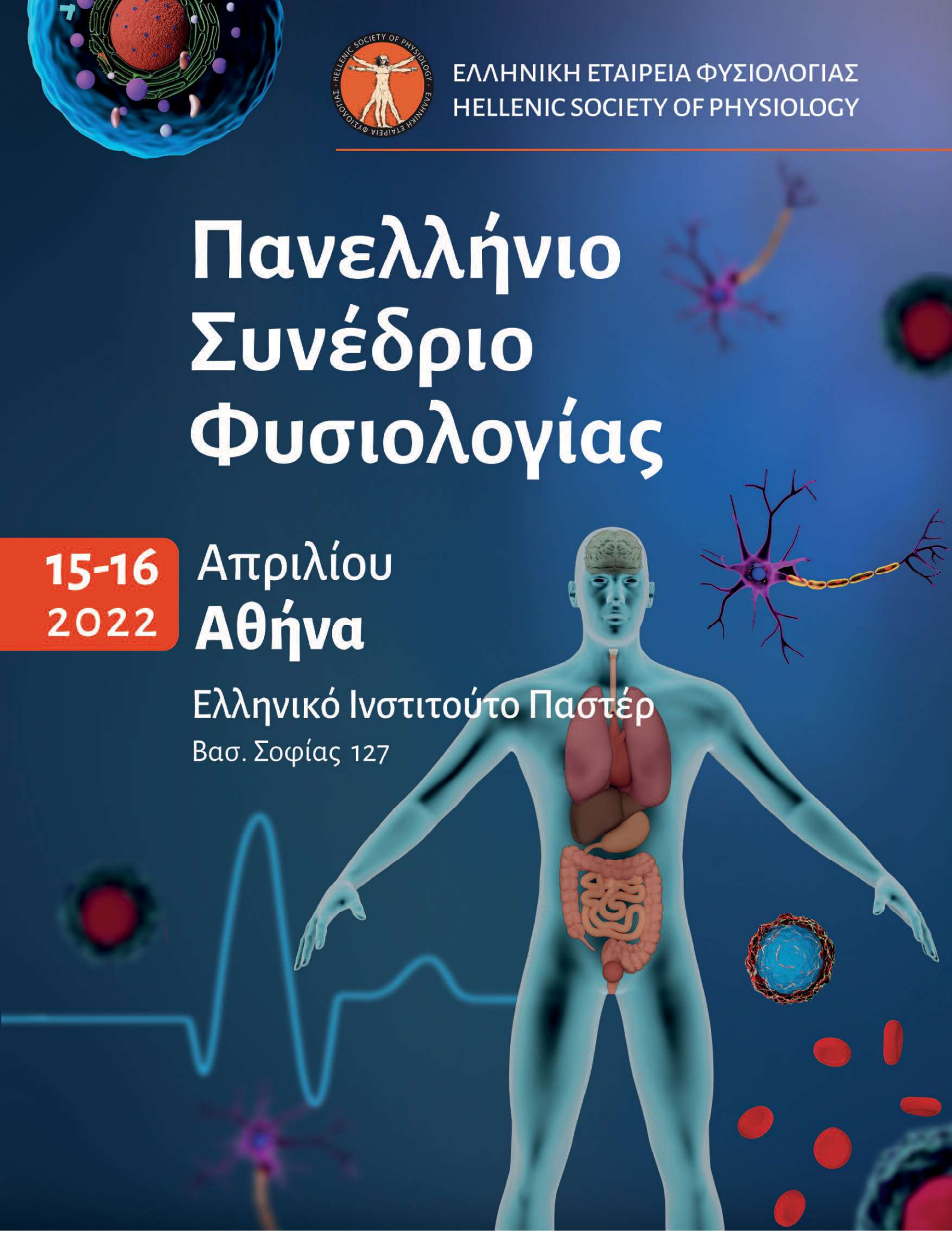
ΕΛΛΗΝΙΚΗ ΕΤΑΙΡΕΙΑ ΦΥΣΙΟΛΟΓΙΑΣ
HELLENIC SOCIETY OF PHYSIOLOGY

Πανελλήνιο Συνέδριο Φυσιολογίας

15-16 Απριλίου 2022

Αθήνα

Ελληνικό Ινστιτούτο Παστέρ
Βασ. Σοφίας 127



ΣΥΝΕΔΡΙΟ
ΕΛΛΗΝΙΚΗΣ ΕΤΑΙΡΕΙΑΣ ΦΥΣΙΟΛΟΓΙΑΣ
2022

ΕΛΛΗΝΙΚΟ ΙΝΣΤΙΤΟΥΤΟ ΠΑΣΤΕΡ
ΑΘΗΝΑ 15-16 ΑΠΡΙΛΙΟΥ 2022

ΠΡΟΓΡΑΜΜΑ

Παρασκευή 15 Απριλίου	
9:00-11:00	**Συνεδρία 1η** **Προεδρείο:** Δ. Πέσχος & Π. Βεζυράκη **Ομιλητές** Μαραζιώτη Αντωνία, Επίκουρη Καθηγήτρια, Τμήμα Φυσικοθεραπείας, Σχολή Επιστημών Υγείας, Πανεπιστήμιο Πελοποννήσου Τίτλος: Η ενδουπεζωκοτική χορήγηση νανοφορέων φαρμάκων ως αποτελεσματική θεραπευτική προσέγγιση του κακοήθους μεσοθηλιώματος Νικόλαος Γιαννακούρης, Αναπληρωτής Καθηγητής, Τμήμα Επιστήμης Διαιτολογίας-Διατροφής, Σχολή Επιστημών Υγείας και Αγωγής, Χαροκόπειο Πανεπιστήμιο. Τίτλος: Διατροφή και Αναπαραγωγική Υγεία Κωνσταντίνος Τσάμης, Επίκουρος Καθηγητής, Ιατρική Σχολή, Πανεπιστήμιο Ιωαννίνων Τίτλος: Στοχευμένη μεταφορά φαρμακευτικών παραγόντων στον εγκέφαλο με τη χρήση νανομορίων για την αντιμετώπιση της νόσου Alzheimer Επιλεγμένη ομιλία από περιλήψεις (#52) Ειρήνη Παπανικολάου, Εργαστήριο Φυσιολογίας και Τομέας Νεφρολογίας, Τμήμα Ιατρικής, Πανεπιστήμιο Ιωαννίνων Υποσχόμενα νανοϋλικά με βάση το γραφένιο για βιοϊατρικές εφαρμογές: μια in vitro μελέτη αξιολόγησης τοξικότητας
11:00-11:30	**Διάλειμμα**
11:30-13:00	**Συνεδρία 2η** **Προεδρείο:** Σ. Ευθυμιόπουλος & Ρ Τέντα **Ομιλητές** Κωνσταντίνος Χατζηστέργος, Αναπληρωτής Καθηγητής, Τμήμα Βιολογίας ΑΠΘ Τίτλος: Μηχανισμοί Ανάπτυξης και αναγέννησης καρδιακών μυοκυττάρων Γεώργιος Καραρήγας, Καθηγητής, School of Health Sciences, Faculty of Medicine, University of Iceland Τίτλος: Φυλοειδικοί μηχανισμοί στην (παθο)φυσιολογία της καρδιάς Επιλεγμένη ομιλία από περιλήψεις (#7) Δήμητρα Παλιούρα, Εργ. Φυσιολογίας Ζώων, Τμήμα Βιολογίας, ΑΠΘ Η ενεργοποίηση του PPARβ/δ αποτρέπει τον εκφυλισμό των μιτοχονδρίων, την ανάπτυξη φλεγμονής και ίνωσης σε ένα γενετικό ζωικό πρότυπο καρδιακής ανεπάρκειας
13:00-15:00	**Αναρτημένες ανακοινώσεις 1, Ελαφρύ γεύμα** **Θα παρουσιαστούν οι ανακοινώσεις 1- 32**

15:00-16:30	**Συνεδρία 3η** **Προεδρείο:** Μ Μαριδάκη & Ε Σπάνδου **Ομιλητές** Ευφροσύνη Παρασκευά, Καθηγήτρια, Τμήμα Ιατρικής, Σχολή Επιστημών Υγείας, Πανεπιστήμιο Θεσσαλίας Τίτλος: Μεταβολική προσαρμογή των καρκινικών κυττάρων στην υποξία: Ρύθμιση της σύνθεσης τριγλυκεριδίων από τον HIF-1. Σωτήριος Ζαρογιάννης, Αναπληρωτής Καθηγητής, Τμήμα Ιατρικής, Σχολή Επιστημών Υγείας, Πανεπιστήμιο Θεσσαλίας Τίτλος: Η χρήση της Drosophila melanogaster ως πειραματικό μοντέλο μελέτης των λειτουργικών επιπτώσεων της έκθεσης σε περιβαλλοντικούς παράγοντες. Επιλεγμένη ομιλία από περιλήψεις (#47) Γρηγόριος Παπαδόπουλος, Εργαστήριο Φυσιολογίας, Ιατρική Σχολή, ΕΚΠΑ Ενοποιημένη πρωτεομική και λιπιδομική ανάλυση της μη-αλκοολικής λιπώδους ηπατικής νόσου που προκαλείται από υψηλής φρουκτόζης corn syrup κατά την παχυσαρκία
16:30-17.30	**Συνεδρία 4η** **ΕΝΗΜΕΡΩΣΗ ΚΑΙ ΠΑΡΟΥΣΙΑΣΗ ΤΩΝ ΠΡΟΓΡΑΜΜΑΤΩΝ / ΥΠΟΤΡΟΦΙΩΝ ΤΟΥ ΙΚΥ / ERASMUS** **Προεδρείο:** Μ. Κουτσιλιέρης **Ομιλητές** Ειρήνη Ντρούτσα, Προϊσταμένη Διεύθυνσης Υποτροφιών ΙΚΥ Τίτλος: Τα προγράμματα προπτυχιακών και μεταπτυχιακών υποτροφιών ΙΚΥ Λεωνίδας Παπαστεργίου, Προϊστάμενος Διεύθυνσης Διοίκησης και Οικονομικών Τίτλος: Προγράμματα υποτροφιών κληροδοτημάτων ΙΚΥ Μαρία Νικητάκη, Προϊσταμένη Διεύθυνσης Ειδικών Προγραμμάτων Διεθνών Υποτροφιών ΙΚΥ/ ΕΜΣ ERASMUS+ Τίτλος: Προγράμματα μετακινήσεων και συμπράξεων ΙΚΥ/ERASMUS+
17:30-18:00	Διάλειμμα
18:00–19:00	**Συνεδρία 5η** **Προεδρείο:** Α Λάζου & Α. Χατζηγεωργίου **Ομιλητές** Σταματία Παπουτσοπούλου, Επίκουρη Καθηγήτρια, Τμήμα Βιοχημείας και Βιοτεχνολογίας, Σχολή Επιστημών Υγείας, Πανεπιστήμιο Θεσσαλίας Τίτλος: Ανοσολογική προσέγγιση της φλεγμονώδους νόσου του εντέρου Μιχάλης Βερυκοκάκης, Ερευνητής Γ, Ερευνητικό Κέντρο Βιοϊατρικών Επιστημών "Αλέξανδρος Φλέμινγκ Τίτλος: Μεταγραφική ρύθμιση της ανάπτυξης των φυσικών Τ-λεμφοκυττάρων

	Επιλεγμένη ομιλία από περιλήψεις (#22) Μαρία Σάκκου, Κέντρο Νέων Βιοτεχνολογιών και Ιατρικής Ακριβείας, ΕΚΠΑ και ΕΚΕΒΕ «Αλέξανδρος Φλέμινγκ» Single-cell δυναμική χρωματίνης και μεταγραφώματος των αρθρικών ινοβλαστών κατά την μετάβαση από την ομοιόσταση στην παθολογία στην αρθρίτιδα που προκαλείται από TNF
19:00-19:30	**Τελετή έναρξης** Χαιρετισμοί
19:30-20:30	**Κεντρική ομιλία** **Προεδρείο:** Μ. Κουτσιλιέρης & Σ. Ταραβήρας **Ομιλητής** **Γεώργιος Κόλλιας** Καθηγητής, Ιατρική Σχολή, ΕΚΠΑ, Μέλος της Ακαδημίας Αθηνών. Τίτλος: TBA

Σάββατο 16 Απριλίου	
9:00-11:00	**Συνεδρία 6η** **Προεδρείο:** Ε. Δούδα & Χ. Ευαγγελινού **Ομιλητές** Χριστίνα Καρατζαφέρη, Καθηγήτρια, ΣΕΦΑΑ, Πανεπιστήμιο Θεσσαλίας Τίτλος: Σκελετικός Μυς: κρίσιμες φυσιολογικές παράμετροι στην υγεία και την απόδοση Μαρία Κοσκολού, Αναπληρώτρια Καθηγήτρια, ΣΕΦΑΑ, ΕΚΠΑ Τίτλος: Φυσιολογικές αποκρίσεις και απόδοση αναιμικών ατόμων κατά την άσκηση σε Υποξία Αναστάσιος Φιλίππου, Αναπληρωτής Καθηγητής, Ιατρική Σχολή, ΕΚΠΑ “Ρύθμιση της έκφρασης της ισομορφής IGF-1Ec στη βλάβη και αναγέννηση του σκελετικού μυός’’ Επιλεγμένη ομιλία από περιλήψεις (#60) Ο. Δαμιανίδου, Εργ. Πειραματικής Νευρολογίας και Εργ. Πειραματικής Φυσιολογίας, Τμήμα Ιατρικής, ΑΠΘ Ανοσολογικές αποκρίσεις ενάντια προδρόμων νευρικών κυττάρων μετά την μεταμόσχευση σε πειραματικό ζωικό πρότυπο πολλαπλής σκλήρυνσης
11:00-11:30	**Διάλειμμα**

11:30-13:30	**Συνεδρία 7η** **Προεδρείο:** Μ. Αλμπάνη & Κ. Ψαροπούλου **Ομιλητές** Ευστράτιος Κοσμίδης, Τμήμα Ιατρικής, ΑΠΘ Τίτλος: Μελέτη πολύπλοκων συστημάτων: Οπτογενετική και Μοντελοποίηση Βασίλης Ράος, Ιατρική Σχολή, Πανεπιστήμιο Κρήτης Τίτλος: Περί των κινητικών και γνωσιακών λειτουργιών του προκινητικού φλοιού. Αποστολία Χατζηευθυμίου, Αναπληρώτρια Καθηγήτρια, Τμήμα Ιατρικής, Σχολή Επιστημών Υγείας, Πανεπιστήμιο Θεσσαλίας Τίτλος: Επιληπτογένεση Επιλεγμένη ομιλία από περιλήψεις (#58) Τατιανή Κουκοβίνη, Τμήμα Βιολογικών Εφαρμογών, Πανεπιστήμιο Ιωαννίνων Προγεννητική έκθεση σε αλκοόλ και σοβαρότητα επιληπτικών κρίσεων σε αναπτυσσόμενους και ενήλικες επίμυες.
13:30-15:00	**Αναρτημένες ανακοινώσεις 2, Ελαφρύ γεύμα** **Θα παρουσιαστούν οι ανακοινώσεις 33- 63**
15:00-16:00	**Συνεδρία 8η** **Νέες τεχνολογίες στην Φυσιολογία και την Έρευνα** **Προεδρείο:** Ο. Παγωνοπούλου & Α Φιλίππου **Ομιλητές** Ομιλία Antisel (sponsored) Δημήτρης Μπουγιουκλής Τίτλος: Κυτταρομετρία ροής και ανάλυση φάσματος: εφαρμογή στην ερευνητική πρακτική Dr. Ελένη-Κυριακή Βέτσικα Ελληνική Ερευνητική Υποδομή για την Εξατομικευμένη Ιατρική, Ιατρική Σχολή, ΕΚΠΑ Τίτλος: Mass Cytometry (CyTOF): Εφαρμογές στην έρευνα της Φυσιολογίας
16:00-18:00	**Συνεδρία 9η** **Προεδρείο:** Β. Ασημακόπουλος & Ι. Ταϊτζόγλου **Ομιλητές** Ιωάννης Γεωργίου Καθηγητής, Τμήμα Ιατρικής, Πανεπιστήμιο Ιωαννίνων Τίτλος: Η Επιστήμη της Ανθρώπινης Αναπαραγωγής Μάρα Σιμοπούλου Αναπληρώτρια Καθηγήτρια, Ιατρική Σχολή, ΕΚΠΑ Τίτλος: Αναπαραγωγικό Δυναμικό: Ισορροπώντας ανάμεσα στη Φυσιολογία και στις Σύγχρονες Εξελίξεις της Υποβοηθούμενης Αναπαραγωγής

	Μαρία Τσανταρλιώτου Καθηγήτρια, Τμήμα Κτηνιατρικής, ΑΠΘ Τίτλος: In Vitro Production: η εφαρμογή των τεχνικών υποβοηθούμενης αναπαραγωγής στα ζώα Επιλεγμένη ομιλία από περιλήψεις (#20) Μαρία Βενετικίδου, Τμήμα Μοριακής Βιολογίας και Γενετικής, ΔΠΘ Διερεύνηση των κυτταροπροστατευτικών μηχανισμών της ALDH3A1 στο επιθήλιο του κερατοειδούς
18:00-18:30	**Διάλειμμα**
18:30-19:30	**Κεντρική Ομιλία** **Προεδρείο** Κ. Μαυραγάνη & Σ. Ταραβήρας **Ομιλητής** **Benedikt Berninger, Καθηγητής, Center for Developmental Neurobiology, King's College London, UK** Τίτλος: Δημιουργία in vivo ενδιάμεσων νευρώνων μετά από in vivo επαναπρογραμματισμό
19:30-20:00	**Τελετή λήξης – Ανακοίνωση Βραβείων**

Κυριακή 17 Απριλίου	
10:00-12:00	ΔΣ ΕΕΦ

ΠΕΡΙΛΗΨΕΙΣ
ΠΡΟΦΟΡΙΚΩΝ ΚΑΙ ΑΝΑΡΤΗΜΕΝΩΝ
ΑΝΑΚΟΙΝΩΣΕΩΝ

1. Exercise-induced effects on Liver Steatosis Indices and Glycemic Profile In NAFLD Patients

Dimitrios Voudouris, Zoi Apostolopoulou, Maria Horianopoulou, Costas Chryssanthopoulos, Michael Koutsilieris, Anastassios Philippou*
*Corresponding author"
Medical School - National and Kapodistrian University of Athens, Athens, Greece "Abstract

Long-term aerobic exercise, either as an exclusive intervention or in combination with resistance exercise, has been shown to have beneficial effects in patients with non-alcoholic fatty liver disease (NAFLD). The aim of this study was to investigate the effectiveness of a short-term, combined exercise program that was performed daily on liver steatosis indices and glycemic profile of patients with NAFLD.

Twenty adult volunteers, twelve males and eight females participated in the study after performing liver ultrasound to document the NAFLD. Baseline and post-intervention assessment included: body weight (BW), waist circumference (WC), Hip circumference (HC), Hip/Waist Ratio (H/W), (HOMA-IR), serum insulin, serum glucose, blood lipids and steatosis indices. Participants followed a combined, aerobic and resistance exercise protocol for 7 consecutive days. Throughout the intervention protocol participants were advised not to change their dietary habits or engage in any form of regular physical activity other than the activities of daily living.

Comparing the values before and after intervention, the following levels of significance (p) and the corresponding effect sizes were recorded: fasting serum glucose p=0.45 (0.17); fasting serum insulin p=0.33 (0.23); HOMA-IR index (indirect insulin sensitivity index) p=0.49 (0. 46); Triglycerides p=0.03 (0.50); Cholesterol p=0.03 (0.50); HSI p=0.23 (0.28); FLI p=0.01 (0.63); LAP p=0.01 (0.65), WC p=0.00 (1.67); HC p=0.007 (0.42); H/W p=0.00 (0.92); BW p=0.39 (0.20). We conclude that the 7-day combined exercise program led to beneficial effects regarding hepatic steatosis and somatometric parameters in patients with NAFLD without any change in BW. Lack of significant changes in glycemic indexes after the completion of the exercise program imply that the observed positive effects are not associated with a simultaneous change in the glycemic profile of these patients."

2. Scleroderma-specific autoantibodies: Should they be included in the diagnostic work-up for Sjögren's syndrome?

Nikolaos Marketos(1), Vasiliki Koulouri(1), Evangelia P. Piperi(2), Maria E. Georgaki(2), Nikolaos G. Nikitakis(2), Clio P. Mavragani(1,3)

1) Department of Physiology, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece

2) Department of Oral Medicine & Pathology and Hospital Dentistry, School of Dentistry, National and Kapodistrian University of Athens, Athens, Greece

3) Joint Academic Rheumatology Program, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece

"Objectives: Sicca complaints are a frequent reason for rheumatologic consultation. Testing for specific antibodies against Ro/SSA and La/SSB antigens and minor salivary gland (MSG) biopsy are among the main tools implemented in the diagnostic work-up. Anticentromere antibodies and sicca manifestations are frequently detected in Sjögren's syndrome (SS) and systemic sclerosis (SSc), respectively. Herein, we aimed to determine the frequency and clinical associations of a wide spectrum of scleroderma (SSc)-specific autoantibodies in consecutive patients referred for evaluation of possible SS.

Methods: Demographic, clinicopathological, and laboratory data were recorded in 216 consecutive patients with sicca complaints. All study participants were tested for SSc-specific autoantibodies (against CENP, PM/Scl, Scl-70, Ku, NOR90, RP11, RP155, fibrillarin, PDGFR, and Th/To) using a commercially available immunoblot kit. According to band intensity, the identified autoantibodies were further classified in those with strong and medium titers.

Results: SSc-specific autoantibodies were detected in 41.7% (90/216) patients evaluated (19% at strong, 22.7% at medium titers) without significant differences between anti-Ro/SSA positive and negative groups. At strong titers was significantly higher in patients with MSG biopsies fulfilling SS histopathological criteria (30% vs 12.5%, p=0.009). This association remained significant after adjustment for antibodies against Ro/SSA and La/SSB autoantigens [OR 95% (CI): 4.1 (1.5-10.6)].

Conclusion: SSc-specific autoantibodies are frequently detected among patients presenting with sicca complaints and at strong but not medium titers are independently associated with MSG biopsy positivity. Taken together, these data imply a useful role of SSc antibody testing in the diagnostic work-up and possibly in the classification criteria for SS.

3. Lysosomal stress driven by mTOR inhibition downstream of IFNAR1 controls dendritic cell type 1 fate in tumors.

1,2 E. Aerakis, 1A. Chatzigeorgiou, 1D. Kerdidani, 1 I. Angelidis, 1M. Matthaiakaki-Panagiotaki , 3S. Henri and 1M. Tsoumakidou. "
1Institute of Bioinnovation, BSRC Alexander Fleming, Vari, Greece
2Laboratory of Physiology, Medical School, National and Kapodistrian University of Athens, Greece
3 Centre d'Immunologie de Marseille-Luminy, Aix Marseille Université, INSERM, CNRS, Marseille, France"

Migratory XCR1+CD11b- conventional dendritic cells type 1 (cDC1s) and XCR1-CD11b+ cDC2s are distinct, spatiotemporally regulated, professional antigen presenting cells. In tumors, they may converge in a common XCR1lowCD11b+ dysfunctional mature-regulatory state. For poorly understood reasons bona-fide XCR1+CD11b- cDC1 are particularly scarce within tumors. We aimed to interrogate the gene expression and functional modules that control cDC1 fate in tumors. Leveraging two cDC1-dependent orthotopic lung cancer models we profiled the transcriptome of tumor versus healthy lung XCR1+CD11b- cDC1s via bulk RNAseq. Differential expression and pathway analysis suggested that tumor cDCs1 have up-regulated genes associated with lysosomal stress and their states are largely driven by interferons (IFNs). Comparative analysis of public murine and human scRNAseq datasets pointed to a shared tumor-induced, IFN-regulated cDC1 cluster with prominent lysosomal activities. Imaging, tracking of acidic organelles and monitoring of antigen degradation confirmed the lysosomal hyperactivity of primary human and murine lung tumor cDCs1 compared to those of healthy lungs. The same state was induced in the mutu1940 cDC1 cell line upon exposure to tumor homogenate, tumor culture medium or IFNs, predominately of type I. Inhibition and activation assays pointed to a previously unknown link between type 1 IFNs, mTOR inhibition and apoptosis. Intriguingly, tumor-infiltrating cDC1s showed higher apoptosis rates compared to healthy lung cDC1s. In mixed IFNAR1.KO/wild-type bone-marrow chimeras, tumor-infiltrating IFNAR1KO cDC1s were protected from mTOR inhibition, lysosome activation and apoptosis. Collectively, we have discovered a regulatory INFRA1-mTOR pathway that is activated in cDC1s in tumors and might render them susceptible to apoptosis. We are currently exploring a mechanistic link between type IFNs –lysosome-dependent cell death and cDC1 paucity in tumors.

4. Transcription factor HIF-1α limits cardiomyoblast proliferation in a pluripotent stem cell model of human cardiomyogenesis.

Angeliki Daiou1, Katerina Petalidou1, Polyxeni-Panagiota Sarri1, Eleftherios I. Papadopoulos1, Georgios Siokatas1, Thomai Mouskeftara2, Efthimios Tsivoglou1, Christine Kottaridi1, Helen G. Gika2, Jeffim N. Kuznetsov3,4, Derek Dykxhoorn5,6, Joshua M. Hare3,5, Antigone Lazou7, Konstantinos E. Hatzistergos1,3,8*.

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

2 Biomic_Auth, Bioanalysis and Omics Lab, Centre for Interdisciplinary Research of Aristotle University of Thessaloniki, Innovation Area of Thessaloniki, Greece

3Interdisciplinary Stem Cell Institute and Departments of 4Ophthalmology, 5Medicine,

6Human Genetics and 8Cell Biology, University of Miami Miller School of Medicine, Miami, FL, USA

7Laboratory of Animal Physiology, Department of Zoology, School of Biology, Aristotle University of Thessaloniki, Thessaloniki, Greece.

Background: The mechanisms controlling cardiomyocyte proliferation in development and regeneration are not well understood. Recent studies link cardiomyocyte proliferation to transient induction of glycolysis, through hypoxia inducible factor 1α (HIF-1α). However, whether induction of glycolysis by HIF-1α is oxygen-dependent, and whether it promotes or represses cardiomyocyte proliferation, remain controversial.

Objectives: To determine the role of HIF-1α in human cardiac myogenesis.

Methods: Using CRISPR-Cas9, we knocked-out HIF-1α in human induced pluripotent stem cells (HIF1α-KO hiPSCs). Wild type (WT) and HIF1α-KO hiPSCs were subjected to chemically defined, stage-specific cardiomyocyte differentiation. Metabolic profiles were studied through gas chromatography and mass spectrometry (GC-MS). Cell-cycle activity was evaluated by flow cytometry, confocal microscopy, and immunoblotting.

Results: Immunoblotting and confocal analyses demonstrated robust, oxygen-independent induction and nuclear translocation of HIF-1α in wild type (WT), but not in HIF1α-KO, hiPSC-derivatives. HIF-1α stabilization peaked on day 7 of differentiation and decreased thereafter. GC-MS showed increased lactate ($p=0.004$) and decreased D-Glucose ($p=0.02$) extracellular levels in WT vs. HIF1α-KO hiPSC-derived cardiomyocytes. In addition, the extracellular concentrations of several Krebs cycle intermediates, with known prolyl hydroxylase domain (PHD) inhibitor capacity, were significantly increased in WT vs HIF1α-KO cells ($p<0.05$). Flow cytometric analysis of cell-cycle with propidium iodide demonstrated ~43% increase in G2M content on day 7 HIF1α-KO vs WT hiPSC-cardiomyoblasts. The enhanced proliferation of HIF1α-KO cardiomyoblasts was confirmed further through proliferating cell

nuclear antigen (PCNA) immunoblotting; as well as via quantitative confocal analysis of the mitotic marker serine-10 phosphorylated Histone H3 ($p<0.001$). Conclusions: Collectively, our results indicate that, during normal human cardiomyogenesis, HIF-1α is transiently stabilized through oxygen-independent mechanisms involving inhibition of PHDs by Krebs cycle intermediates, and shifts cellular metabolism toward aerobic glycolysis to limit cardiomyoblast proliferation. These findings have important therapeutic implications for stimulating cardiomyocyte regeneration in response to heart disease.

5. The effect of muscle blood flow restriction on hemodynamics, cerebral oxygenation, and activation at rest

Evgenia D. Cherouveim1,2, Panagiotis G. Miliotis1, Konstantina Dipla3, Maria D. Koskolou1, Ioannis S. Vrabas3 and Nikolaos D. Geladas1

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The aim of this study was to investigate the effects of muscle blood flow restriction on the hemodynamic responses, muscle and cerebral oxygenation, and cerebral activation at rest. In 26 healthy males, aged 33±2 yrs, physiological variables were continuously recorded during a 10-min period in two experimental conditions: a) with muscle blood flow restriction through thigh cuffs application inflated at 120 mmHg (With Cuffs, WC) and b) without restriction (No Cuffs, NC). Muscle and cerebral oxygenation were reduced by muscle blood flow restriction as suggested by the increase in both muscle and cerebral deoxygenated hemoglobin (Δ[HHb]; $p<0.01$) and the decrease of muscle and cerebral oxygenation index (Δ[HbDiff]; $p<0.01$). Hemodynamic responses were not affected by such muscle blood flow restriction, whereas baroreflex sensitivity was reduced ($p=0.009$). The perception of leg discomfort was higher ($p<0.001$) in the WC than in the NC condition. This study suggests that thigh cuffs application inflated at 120 mmHg is an effective method to reduce muscle oxygenation at rest. These changes at the muscular level seem to be sensed by the central nervous system, evoking alterations in cerebral oxygenation and baroreflex sensitivity.

6. Effect of Short-term Metabolic Insults on the Rat Hippocampus - An ex vivo Study

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Ischemia is a metabolic disorder in which blood flow to a tissue is restricted resulting in reduced access to oxygen and metabolic substrates and increased metabolite accumulation in tissues. Depending on the duration and severity of ischemia, tissue morphology can be altered and/or necrosis may occur. In previous experiments we have shown that ischemia (oxygen & glucose deprivation) hypoxia (oxygen deprivation) and hypoglycaemia (glucose deprivation) have a differential effect on glutamate and GABA release from the rat retina, while ischemia leads to a significant increase of both neurotransmitters' release from the rat hippocampus.
The aim of this study was to assess the effects of ischemia, hypoxia and hypoglycaemia on hippocampal morphology. The protocol followed matched the neurotransmitter release experiments mentioned above. After 1h of pre-superfusion 250μm hippocampal slices were superfused for 26min with oxygenated glucose-artificial cerebrospinal fluid (arti-CSF; 1.2ml/min) and for 66min with arti-CSF matching the desired metabolic insult. Subsequently, tissues were fixed, cryoprotected, snap-frosted and stored at -80oC. Finally, 10μm sections were histochemically proceeded using Cresyl Violet (Nissl bodies: rough endoplasmic reticulum and ribosomes) and 4′,6-diamidino-2-phenylindole (DAPI; cell nuclei) stains.
According to our results, morphological changes were observed in the pyramidal cell layer of the CA1 and CA3 fields under ischemic and to a lesser extent under hypoglycaemic and hypoxic conditions. Under ischemic conditions a statistical significant decrease of Nissl bodies and cell nuclei number was observed in the pyramidal cell layer of the CA1 field.
The results of this study, suggest that short-term ischemia, hypoxia and hypoglycaemia affect in a differential way the rat hippocampus, and that, as expected, ischemia has the most noticeable effect. Our results are in agreement with previous studies (Medvedeva et al., 2017, doi: 10.1523/JNEUROSCI.3270-16.2016) concerning the prominent susceptibility of the CA1 hippocampal field to ischemia compared to the CA3 field and dentate gyrus."

SELECTED FOR ORAL PRESENTATION

7. PPARβ/δ activation prevents mitochondrial degeneration, inflammation and fibrosis in a genetic animal model of heart failure

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PPARβ/δ is a major transcription regulator of cardiac energy metabolism with anti-inflammatory, anti-oxidative properties and cardioprotective action. In this study, we sought to investigate whether pharmacological activation of PPARβ/δ could ameliorate cardiac tissue damage in desmin null mice (Des-/-), a genetic model of heart failure and explore the potential effects on the impaired mitochondrial network. Damaged mitochondria, extensive cardiomyocyte death, an early acute inflammatory response and severe cardiac remodeling lead to dilated cardiomyopathy and eventually heart failure in Des-/- mice. Our findings demonstrate that PPARβ/δ activation prevents the development of severe cardiac inflammation, fibrosis and cardiac remodeling, all hallmarks of the Des-/- heart. Furthermore, PPARβ/δ activation alleviates oxidative stress in the failing myocardium as evidenced by decreased superoxide levels. Importantly, PPARβ/δ activation stimulates mitochondrial biogenesis, protects mitochondria from vacuolar degeneration and improves the deranged mitochondrial network as observed in transmission electron microscopy images. Concomitantly, PPARβ/δ promotes the fission/fusion balance and enhances mitochondrial functionality in Des-/- hearts. In conclusion, PPARβ/δ activation exerts protective effects during myocardial degeneration and heart failure in Des-/- hearts by preserving the overall structural and functional quality of the mitochondrial network and attenuating inflammation and fibrosis. These findings implicate PPARβ/δ for further clinical development.

8. Activation of nuclear receptor PPARβ/δ improves mitochondrial respiratory function during myocardial I/R

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Activation of nuclear receptor peroxisome proliferator activated receptor β/δ (PPARβ/δ) confers cardioprotection via pleiotropic antioxidant and anti-inflammatory actions. However, the mechanisms underlying PPARβ/δ-mediated effects during myocardial ischemia/reperfusion (I/R) are not yet fully elucidated. The aim of this study was to investigate the effect of PPARβ/δ activation on mitochondrial respiratory function during. For this purpose, the PPARβ/δ agonist GW0742 and/or antagonist GSK0660 were administered to rats in vivo and mitochondrial respiration and ROS production rates were determined using high resolution fluorespirometry. Heart tissues were then used for determination of expression of components of FAO-linked respiration pathway and transcription factors governing mitochondrial respiratory capacity and biogenesis. Activation of PPARβ/δ reduced mitochondrial ROS production during in vitro anoxia/reoxygenation and improved fatty acid oxidation (FAO)-dependent mitochondrial respiration rate and ROS production at oxidative phosphorylation (OXPHOS)-dependent state during ex vivo myocardial I/R. PPARβ/δ activation was also accompanied by increased mRNA expression of carnitine palmitoyl transferase 1b and 2 (CPT-1b, CPT-2), electron transfer flavoprotein dehydrogenase (ETFDH), peroxisome proliferator activated receptor gamma co-activator 1 alpha (PGC-1α), nuclear respiratory factor 1 (NRF1) and succinate dehydrogenase A (SDHA). Finally, increased citrate synthase (CS) activity and preservation of cardiac ATP content post-I/R were observed. In conclusion, activation of PPARβ/δ improves mitochondrial respiratory function during I/R through stimulation of both FAO-linked respiration and PGC-1α/ NRF1 signaling, resulting in cardioprotection.

9. Investigation of the role of the centriolar satellite protein, OFD1, in the development of the human cerebral cortex and its malformations using animal models

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Development of the brain is a highly orchestrated process depending on the correct balance of neural progenitors' proliferation and their differentiation into neurons and glial cells. In animal models, primary cilia have been shown to be critical for the development of the brain, while mutations in genes controlling cilia formation and function lead to ciliopathies which are associated with brain malformations and dysfunction. Preliminary data in rodents have been used for deciphering the role of primary cilia in brain development and its malformations, however their role remains unclear. OFD1, a cilia-related gene encoding a centrosomal protein, was found mutated both in the ciliopathy orofacial digital type 1 syndrome and the cortical malformation periventricular heterotopia. Focusing on OFD1, our study aims to shed light on the role of primary cilia in mechanisms regulating normal cortical development and MCD in humans. We examined temporal, spatial and species-specific expression of OFD1 from bulk and single-cell transcriptomic data to identify its expression profile and potential differences across different cell types during brain development. We subsequently performed ectopic OFD1-overexpression (OE) or silencing in the developing cerebral cortex of wild type mouse embryos via in utero electroporation to investigate its role in vivo. We used a panel of fluorescent antibodies to examine potential differences between the distinct cell types comprising the cortex and we observed that during OFD1 OE there is an increase of neural progenitors. Finally, to model human brain disorders, we will generate either knock-out or mutated OFD1 cerebral organoids as found in patients with brain malformations. Our aim is to compare these findings with those from our in vivo mouse analysis and contribute to modelling brain disorders due to the malfunction of primary cilia.

10. Drosophila melanogaster models of Sestrin-associated human lung diseases: In Silico analysis of the fruit fly Sestrin interactome.

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Introduction: Human Sestrins (Sesns) are stress-inducible proteins that regulate oxidative stress, inflammation, autophagy, endoplasmic reticulum stress, and metabolic homeostasis. Growing evidence has supported the involvement of Sesns in the pathogenesis of lung diseases such as emphysema and lung cancer (Wang et al. Cell Physiol Biochem 2017; 43:1731-1741). In this study, by using an in-silico approach we investigated the Sesn protein interaction network in the fruit fly which could be used as an animal model of Sesn-associated human lung diseases. Materials and Methods: The Drosophila Sesn interaction network was identified through the String v 11.0 database. Functional enrichment analysis (FEA) relative to biological process (BP) was performed using the GeneCodis 4.0 tool. Sesn expression profile was created through GEOprofiles. Results: Eleven proteins were found to interact with Sesn: CG6888, Alc, SNF4Agamma, AMPKalpha, RagA, RagB, Nprl2, Nprl3, CG7609, CG4705 and Jafrac1. FEA with respect to BP showed involvement of these proteins to macroautophagy, cell growth and response to amino acid starvation. GEOprofile analysis indicated significant changes in Sesn expression during different developmental stages and after chronic ethanol (EtOH) exposure, that was also accompanied by changes in the expression of Jafrac1, CG7609, and CG4705. Conclusions: In the fruit fly, EtOH-mediated airway impairment induces changes in the expression of Sesn and its interactors. Since chronic EtOH exposure has been associated with the pathogenesis and progression of human pulmonary disease, Drosophila studies of the Sesn interactome could provide insights into relevant mechanisms in humans.

11. The role of genomic instability-induced inflammation in colon carcinogenesis progress

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The maintenance of genome stability is an important procedure for the accurate transmission of genetic information. Thus, it is crucial for DNA replication to underlie strict regulation since cases of its dysfunction lead to development of replication stress, a frequent source of genomic instability that enhances tumorigenesis and is lately considered to influence the innate immune system(1),(2). The replication licensing factor Cdt1 and its inhibitor Geminin are key regulators of DNA replication. According to previous studies of our research group, changes in expression levels of these proteins contribute to the development of murine lung and colon carcinogenesis(3). The present project aims to investigate the mechanism that activates inflammation developed during genomic instability which is induced by the deregulation of Geminin and Cdt1 expression levels. Recent work of an in vivo model of colon carcinogenesis demonstrates the presence of inflammation after tissue-specific deletion of Geminin or/and overexpression of Cdt1.
In this context, we are going to use the colon cancer cell line HCT116 and a chemically induced colon carcinogenesis protocol in transgenic mice in order to pinpoint the molecular pathway that activates inflammation caused by replication stress. One of the long-term goals of the proposed research work is to get insight on how inflammation determines the progress of a cancer, as well as to reveal molecular targets for anticancer therapy.

(1) Petropoulos, Michalis et al. “Replication Licensing Aberrations, Replication Stress, and Genomic Instability.” Trends in biochemical sciences vol. 44,9 (2019): 752-764
(2) Ragu, Sandrine et al. “Replication Stress, DNA Damage, Inflammatory Cytokines and Innate Immune Response.” Genes vol. 11,4 409. 9 Apr. 2020
(3) Champeris Tsaniras, Spyridon et al. “Geminin ablation in vivo enhances tumorigenesis through increased genomic instability.” The Journal of pathology vol. 246,2 (2018): 134-140"

12. Investigation of the role of the ciliary protein AHI1 in human cortical development and in manifestation of cortical malformations

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The cerebral cortex is one of the most complex structures in mammals, and malfunctions during its development lead to severe brain disorders associated with developmental delays and cognitive deficits. Preliminary studies show that primary cilium, a small organelle serving as cellular antenna, is essential for cortical development. Even though no concrete knowledge exists so far regarding human brain development, defects in ciliary function cause a group of human genetic disorders described as ciliopathies, which lead to severe brain abnormalities including cortical malformations. Several genes have been associated with such disorders, like AHI1 a ciliary-related gene originally identified in the ciliopathy Joubert syndrome and in cortical malformation polymicrogyria. However, the exact mechanisms that lead to the manifestation of such disorders are not yet fully understood. Using single-cell RNA sequencing datasets from the developing mouse and human brain and brain organoid cultures we have studied the spatiotemporal, cell-specific and species-specific expression of AHI1 during development. Furthermore, we have provoked ectopic AHI1 overexpression or silencing in the developing mouse cortex via in utero electroporation to dissect the role of primary cilia in vivo. We aim to examine the effect of Ahi1 gene manipulation in processes such as progenitors' proliferation and differentiation and neuronal migration. Specifically, by using cell type-specific markers we will examine the morphology and number of different progenitor cells and neurons. To study the role of primary cilia in human cortical development, gene manipulation of AHI1 will be performed in human brain organoids. Moreover, mutant brain organoids carrying the AHI1 variants found in patients with Joubert syndrome and polymicrogyria will be used as human-specific models for such disorders. The results of this project will give insight into the role of primary cilium in human cortical development as well as the key mechanisms regulating cortical malformation diseases.

13. Differential effects of conventional and biocompatible Peritoneal Dialysis fluids on the electrophysiological properties of mesothelial monolayers in Ussing chambers

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Objectives: Wider implementation of Peritoneal Dialysis (PD) is compromised by the peritoneal injury resulting primarily from the prolonged exposure to PD fluids. One of the two main transport barriers in the peritoneum is the mesothelium coming in direct contact with the PD fluids. Our aim was to understand how different PD fluids affect the mesothelial permeability.

Methods: Mesothelial (MeT-5A) cells were cultured in Snapwell permeable supports to form a monolayer and then were mounted in Ussing chambers. Conventional and biocompatible PD fluids [Dianeal (D) and Balance (B)] were added for a 4-hour incubation. Transmesothelial resistance (RTM, in Ω*cm2) was monitored and paracellular permeability was assessed by diffusion of a 10-kDa FITC dextran from the apical to the basolateral side. Functional data were combined with molecular ones performing Real Time-PCR (using β-actin as reference gene) for CLDN1, CLDN2 and CLDN3 genes to fully assess the mesothelial barrier function.

Results: A 4-hour incubation with D maintains significantly increased RTM (t0: 18.00±2.88, t1: 68.50±3.79, t4: 49.75±2.42, $p<0.001$) and simultaneously induces lower (%) paracellular transport of FITC-dextran (3.99±0.75%, $p<0.05$) compared with Balance (7.09 ± 0.65%). In contrast, incubation with B initially increased the RTM (t0: 28.00±4.32, t1: 43.66±3.48, $p<0.001$), albeit decreased 4 hours later (t4: 24.83±4.49) approaching the initial value of t0. This reduction was also reflected on the fluorescent marker. However, comparing the gene expression of CLDN1 (ΔCtDianeal: 13.68±1.49; ΔCtBalance: 12.81±1.43), CLDN2 (ΔCtDianeal: 15.23±0.21; ΔCtBalance: 14.75±1.02), CLDN3 (ΔCtDianeal: 17.07±1.96; ΔCtBalance: 17.70±2.28) between the PD fluids, no significant difference was found.

Conclusions: The effects of Balance PD fluid alter the mesothelial barrier function less than compared to Dianeal that has acicid pH and high glucose degradation products. Both PD fluids used did not significantly alter the gene expression of paracellular permeability components. Other Tight junction components should be studied to explain the electrophysiological findings."

14. Molecular identification of the primary cilium BBSome complex in pleural mesothelial cells

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Introduction: The primary cilium (PC) is a sensory cell organelle with multiple roles in cellular homeostasis by detecting extracellular cues. The BBSome is a key component of the PC machinery. Aims: We aimed at characterizing the molecular components of the BBSome in the human benign mesothelial cell line, MeT 5A. Methods: The abundance of BBS1, BBS2, BBS4, BBS5, BBS7, BBS8, BBS9 and BBS18 mRNA transcripts in MeT 5A cells was assessed by quantitative real time PCR using b-actin as the housekeeping gene. Total RNA was isolated by TRIzol™ Reagent. 200 ng of RNA were used to synthesize cDNA in a 20 μL reaction volume with the SuperScript™ III first-strand synthesis system. The cDNA was diluted 1:5 with nuclease-free water. Diluted cDNA (8.4 μl) and primers (0.4 μM) were then added in a 20 μl PCR mix (PowerUp™ SYBR™ Green Master Mix), and amplified in the ABI 7300 Real-Time PCR System. The thermal cycling conditions for all genes were: 50°C for 2 min, 95°C for 2 min followed by 40 cycles of 95°C for 15 sec, 55°C for 30 sec and 72°C for 1 min. Results: We did not detect expression of the BBS5 and BBS8 genes. The gene expression of BBS 1,2,4,7,9,18 relative to b-actin (1/ΔCt) was 0.09, 0.14, 0.07, 0.10, 0.07 and 0.14 respectively. Conclusions: BBS2 and BBS18 showed the highest expression in MeT 5A cells. High BBS2 expression was reported to favor survival in malignant pleural mesothelioma patients (Rouka et al., ERJ 2020 56:1135). Further research is warranted to ascertain the functional role of the PC BBSome in the pleural mesothelial cell physiology and it’s relevance to malignant pleural mesothelioma pathophysiology.

15. Exposure of malignant pleural mesothelioma (MPM) cells to malignant pleural fluid in 3D setting, induces differential expression of primary cilium specific acetylated tubulin protein Human malignant

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Introduction: MPM is an aggressive, chemo-resistant malignancy of the pleural cavity. In MPM, Epithelial-to-mesenchymal transition (EMT) is an important mechanism involved in the invasive behavior of the tumor. The aim of this study was to assess whether the exposure of mesothelial and MPM cells to malignant pleural effusion fluid has a biological effect on their contractile EMT behavior.
Materials and Methods: MeT-5A benign mesothelial cells and MSTO, biphasic MPM cells were used in a gel contraction assay with 10% FBS or 10% FBS with mesothelioma diagnosed pleural fluid (PF). Gels were cast in 24 well plates and imaged on a flatbed scanner on day 1, 2 or 3, and contraction was calculated from area of the gels. Gels were subject to western blot procedure. The expression of acetylated tubulin (ac-tuba1a) a component of cell sensory organelle, primary cilium was assessed.
Results and discussion: In MeT-5A cells, contraction until day 2 was significantly increased in PF condition (0.63□0.01, $p<0.001$) compared to control (0.57□0.009), n=13. In MSTO cell, contraction, in PF media (0.65□0.007, $p<0.001$) was significantly greater till day 3 compared to control (0.43□0.02), n=14. Under PF condition ac-tuba1a expression was significantly lower in both cell types. Gel contraction assay is indicative of EMT phenomenon of cells, on a 3D setting. Our findings suggest an effect of malignant pleural fluid on inducing a mesenchymal behavior of the cells.
Conclusions: Our results demonstrate that exposure to malignant PF causes greater contraction. Primary cilium protein ac-tuba1a expression is reduced in cells that undergo EMT."

16. Interferon regulated genes are identified as main markers by next generation sequencing in antiphospholipid syndrome

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Objectives: Antiphospholipid syndrome (APS) is a rare systemic autoimmune disorder characterized by a wide range of vascular and pregnancy manifestations. Its pathogenesis is not yet unravelled. Here, we identify genes that characterize the disease and its major subtypes and have potential therapeutic implications.
Methods: Next-generation RNA sequencing, differential expression and enrichment analysis of whole blood samples from 64 patients with thrombotic primary APS and 32 healthy controls (HC) is performed. The prediction value of these markers is validated through a nested cross-validation machine learning modelling methodology. The deregulated gene lists are searched for interferon regulated genes (IRGs), while the differential expression analysis is also applied on three major APS subgroups (triple aPL positive/negative, recurrent/non-recurrent thrombotic episodes, arterial/venous thrombotic episodes)
Results: 34 genes are found differentially expressed between APS and HC, while 14 out of them are type I and II interferon regulated genes (IRGs). Our machine learning methodology denotes Random Forest as the best modelling method returning ROC AUC equal to 0.77. Comparison between triple antiphospholipid antibodies (aPL) positive patients and HC returns 50 up-regulated genes (30 IRGs), while triple aPL negative subgroup returns 27 up-regulated genes (12 IRGs). The group of patients with recurrent thrombotic episodes reported versus HC returns 42 up-regulated genes (19 IRGs), while non-recurrent returns 17 up-regulated genes (7 IRGs). Finally, venous versus HC returns 23 up-regulated genes (21 IRGs) and arterial versus HC returns 24 up-regulated genes (7 IRGs).
Conclusion: Type-I and II IRGs are the most frequently differentially expressed genes in APS and in disease subgroups of higher severity."

17. Cognitive efficacy of omega-3 fatty acids in Alzheimer's disease: A meta-analysis of randomized controlled trials

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BACKGROUND
Alzheimer's disease (AD) is a neurological degenerative disorder, the most common type of dementia. It is characterized by chronic and progressive cognitive deterioration in several domains of higher cortical function. Preclinical and clinical studies have suggested the potential beneficial role of omega-3 fatty acid (FA) supplementation in AD.
AIM
Our aim was to identify all randomized controlled trials (RCTs) investigating an association between omega-3 FA supplementation and cognitive function in patients with AD, as assessed with the Alzheimer's Disease Assessment Scale-Cognitive (ADAS-Cog) Subscale test.
METHODS
We searched PubMed and grey literature sources for all published RCTs assessing cognition following supplementation of omega-3 FA compared to placebo, until February 2022. Five studies fulfilled the eligibility criteria and were included in the current meta-analysis.
RESULTS
We collected data from 5 trials in a total of 655 patients with AD, assigned either to omega-3 FA supplementation (387 participants) or placebo (268 participants). The outcome measure of cognition, expressed through the ADAS-Cog score, was evaluated. Omega-3 FA compared to placebo led to a non significant impact on the ADAS-Cog score (Mean Difference=0.52, 95% CI [-0.69, 1.73]). The included studies were similar (I2=0, p-value=0.55). The test for overall effect (Z=0.85, p=0.40) confirmed no statistical difference. Omega-3 FAs did not have a significant impact on the cognitive functions of adults with AD.
CONCLUSION
Omega-3 FA supplementation does not seem to confer any significant benefit on cognition for patients with AD. Therefore, treatment of AD with dietary omega-3 FAs should not be recommended. Omega-3 FAs could be beneficial in halting cognitive deterioration in those people at risk of developing AD, and this possibility should be addressed in future studies."

18. Mitochondrial nonlinear disturbances in cardiovascular diseases

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The mitochondria constitute a central regulator of cellular metabolism producing, in the form of ATP via oxidative phosphorylation, about 90% of the cellular energy needs. Mitochondria have their own circular, double-stranded DNA, which encodes 37 genes, 13 for electron transport chain (ETC) polypeptides, 22 for tRNAs needed for the intra-mitochondrial synthesis of the 13 ETC proteins and 2 mt-rRNAs necessary for their synthesis. Any anomalies in mitochondrial DNA or protein sequence may result in defective oxidative phosphorylation and mitochondrial dysfunction associated with the development of various cardiovascular (CV) diseases. This study examined potential associations between mitochondrial DNA mutations and CV diseases. Cardiac tissue and blood samples were collected from seven patients with a history of cardiovascular disease, who underwent surgery or autopsy at a university hospital. Total DNA was extracted from both myocardial tissue specimens and serum by protease-K digestion, phenol/chloroform extraction and ethanol precipitation. Each DNA sample was quantified and subjected to PCR for the amplification of the ND1 gene, which subsequently was screened for mutations. We identified one individual with a homozygous A□G substitution mutation in DNA isolated from cardiac tissue and two individuals heterozygous A□G mutation in DNA isolated from serum. Specifically, Amplicon sequence analysis showed a homozygous A3397G substitution in ND1 (NADH dehydrogenase 1) gene in sample 6AKTis and heterozygous mutation in A3397G in serum samples 9APSe and 8MPSe. The A to G substitution changes the amino acid from methionine (ATA) to tyrosine (GTA) at position 31 of the ND1 gene, which is part of the mitochondrial membrane respiratory chain NADH dehydrogenase (Complex I). Patients identified to have the aforementioned mutation exhibited similar clinical characteristics with severe multiple vessel atherosclerotic lesions and ischaemia-induced cardiovascular dysfunction. Further studies are needed to reveal potential associations between the identified DNA mutation and specific cardiovascular diseases."

19. Anticancer activities of Layered Double Hydroxides (LDH) nanomaterials functionalized with quercetin

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Background Cancer remains still one of the main causes of death, making its treatment a challenging goal. Recent advances in the field of nanotechnology have shown that Layered Double Hydroxides (LDH) based nanosystems might be promising due to their features. Their good biocompatibility, easy surface modification and high chemical stability indicate great potential for cancer therapy.

Aim To determine the cytotoxic activity of MgAl-NO3− LDH (LDH) and quercetin intercalated MgAl-NO3− LDH (Q-LDH) in normal and cancer cells.

Methods The cytotoxic activity of LDH and Q-LDH against human osteosarcoma cells (Saos-2 cells) and mouse embryonic fibroblasts (NIH/3T3 cells) was assessed utilizing 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and clonogenic assay. The ability of the nanoparticles to generate or scavenge reactive oxygen species (ROS) was measured with flow cytometry (2',7'-dichlorofluorescin-diacetate staining).

Results LDH did not have an obvious impact on NIH/3T3 and Saos-2 cell viability. On the other hand, Q-LDH exerted differential effects on these two cell lines and specifically, a dose- and time-dependent reduction of Saos-2 population and only a dose-dependent of NIH/3T3 population. The augmented cytotoxicity of Q-LDH was also verified with the lower ability of cancer cells to form colonies (reduced by 30%). Immobilization of Q-LDH also affected LDH’s ROS scavenging ability.

Conclusion Quercetin intercalated MgAl-NO3− LDH showed enhanced anticancer activity compared with the MgAl-NO3− LDH alone at equivalent amounts. Understanding the molecular pathways activated by the interaction of LDH and functionalized-LDH with different cell types is fundamental for evaluating their future biomedical applications."

SELECTED FOR ORAL PRESENTATION

20. Investigating the cytoprotective mechanisms of ALDH3A1 in corneal epithelium

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Aldehyde dehydrogenase 3A1 (ALDH3A1) is an NAD(P)+-dependent enzyme that catalyzes the oxidation of various aldehydes to their corresponding acids. ALDH3A1 has been characterized as corneal crystallin because of its high abundance in the mammalian corneal epithelium, where it serves antioxidant defense roles via its ability to detoxify by-products of lipid peroxidation. However, ALDH3A1 is also considered to be a multifunctional protein as it is implicated in additional, non-metabolic, homeostatic functions, such as cellular proliferation and differentiation. Previously, we established an isogenic HCE-2 cell line pair differing only in the expression of ALDH3A1 (HCE-2/ALDH3A1 vs. HCE-2/mock cells) and demonstrated its association with the DNA damage response (DDR) pathway. In this study, the molecular mechanisms underlying the cytoprotective role of ALDH3A1 in human corneal epithelial cells (HCE-2) were further investigated under normal, oxidative (H2O2) and genotoxic (etoposide) conditions. The results demonstrated that ALDH3A1 expression is associated with slower proliferation rate and a G2/M accumulation of HCE-2 cells. Furthermore, ALDH3A1 protected corneal epithelial cells from the H2O2 and etoposide-induced apoptosis. Immunofluorescence analysis showed that ALDH3A1 was associated with lower formation of γH2Ax foci and increased expression of p21 following treatment with either H2O2 or etoposide. Finally, flow cytometry analysis revealed a significant increase on the protein levels of p53 as well as on the phosphorylation levels of p53 at Ser15 in the ALDH3A1/HCE-2 cells under treatment conditions compared to control. Taken together, the involvement of ALDH3A1 in the modulation of the DDR pathway may account for some of the cytoprotective properties attributed to ALDH3A1 in the corneal epithelium. The clarification of the role of ALDH3A1 in corneal homeostasis could be crucial for unraveling novel therapeutic strategies, aiming at various corneal pathologies.

21. The effects of graphene oxide (GO) and oxidized carbon nanodiscs (oxCNDs) on normal human fibroblast cells

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Background Carbon materials exhibit unique physical and chemical properties (nanometric dimensions, large specific surface area etc) that make them ideal candidates for carriers in controlled drug release systems. Graphene and carbon nanodiscs (disc-shaped graphene stacked atop each other) are two carbon materials that have attracted interest in nanomedicine. The surface of these materials can be functionalised using the oxidation process which leads to the formation of hydroxyl and carboxyl groups. These groups can then be used to bond different compounds to the carrier (graphene oxide or oxidized carbon nanodiscs).
Aim To examine in vitro the cytotoxic effects of GOs and oxCNDs on normal human fibroblast cells (NIH/3T3 cells).
Methods The cytotoxic activity of GO and oxCNDs against NIH/3T3 was assessed by means of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide-MTT and clonogenic assays. Detection of reactive oxygen species (ROS) was quantified with flow cytometry (2',7'-dichlorofluorescin-diacetate staining).
Results The MTT assay showed a dose-depended cytotoxic activity for both materials. However, lengthening of exposure time to the materials (48 hours) increase the cytotoxicity of oxCNDs as they become more toxic than GO. Flow cytometry revealed a similar endogenous ROS production after treatment with GO or oxCNDs. Even though both nanomaterials disturbed the cellular redox state the long-term survival of cells was lower for those treated with oxCNDs than GO.
Conclusion Our results indicate that GO and oxCNDs act through different toxicology mechanisms against normal cells. Identification of the molecular pathways activated by these two graphene nanomaterials could improve their biological safety and facilitate their wide biomedical and pharmaceutical applications."

SELECTED FOR ORAL PRESENTATION

22. Single-cell chromatin and transcriptome dynamics of Synovial Fibroblasts transitioning from homeostasis to pathology in TNF-driven arthritis

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Synovial Fibroblasts (SFs) are key pathogenic drivers in Rheumatoid Arthritis (RA). They mediate inflammation, cartilage and bone destruction, yet therapies targeting SFs are missing. Our early studies established TNF causality in the full pathogenic process. Indeed, TNF-blockade therapies proved efficacious for a large proportion of RA patients. Recent, single cell (sc) analyses of human RA affected joints revealed the identities of SF subpopulations with distinct functional and anatomical location features. Thy1+ pro-inflammatory subpopulations located in the sublining layer, Thy1- destructive populations in the lining layer, and a Notch3+ subpopulation found in the perivascular space fueling the pathogenic SF pool. However, the homeostatic to pathological transitions of SFs at the cell state and molecular level remain unclear. Here, we aimed to identify homeostatic and pathological functions of SFs, the transcription factors (TFs) and gene regulatory networks (GRNs) that drive the pathogenic process in the human TNF overexpressing (hTNFtg) arthritic mouse model. Thus, sc-RNA-sequencing and sc-ATAC-seq was performed on SFs isolated from healthy and hTNFtg mice. Among the SF subpopulations, we found distinct homeostatic and TNF driven disease-specific states. In healthy joints, Thy1- lining subpopulation mainly regulates repairing and cell death responses, whereas Thy1+ SF subpopulations function towards maintaining joint structures and immune surveillance. Arthritis progression leads to the emergence of distinct disease-specific SF

states exhibiting enhanced inflammatory responses, promigratory behavior, neovascularization and collagen metabolic processes. Transcriptomic and chromatin accessibility data integration revealed that NFkB, Bach1 and Runx1 TFs realize the epigenetic potential of primed Thy1+ subpopulations downstream of TNF. Comparative studies uncovered highly conserved mouse/human profiles at all levels.

Our study provides a map of transcriptomic and epigenomic profiles identifying the TFs and GRNs that remodel SFs identities and functions during onset and progression of arthritis, providing a blueprint for future testing of novel fibroblast-targeted diagnostic and therapeutic modalities for RA."

23. In vitro cytotoxicity of surface-functionalized superparamagnetic iron oxide nanoparticles

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Background: In the last decade, superparamagnetic iron oxide nanoparticles (SPIONs) have attracted the interest of the scientific community due to the multitude of applications in many fields of biomedicine namely as contrast media in diagnosis or as carriers for targeted drug delivery.
Aim: To evaluate the cytotoxic activity of hydrophilic and surface functionalized SPIONs in vitro against normal cells (fibroblasts, NIH/3T3 cells).
Methods: Super-hydrophilic ultra-small SPIONs were produced by ""trimming"" the surface coating of the as-prepared nanoparticles. Cytotoxicity was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and the clonogenic assay. Flow cytometry was employed to study the effect of SPIONs on the redox stage of the cells through the detection of reactive oxygen species (ROS) generation using 2',7'-dichlorofluorescin-diacetate (DCF-DA) and the induction of apoptosis using Annexin V-FITC/Propidium Iodide staining.
Results : SPIONs caused a dose-dependent production of ROS (1–10 μg/ml), possibly leading to apoptotic cell death noted after 48 hours. Consequently, a decrease in NIH/3T3 cell viability was observed after exposing them to increasing concentrations of SPIONs (1–100 μg/ml). Moreover, according to the clonogenic assay SPIONs caused irreversible damages to the cells which failed to grow and form colonies at concentrations lower than 5 μg/ml.
Conclusion: Functionalized SPIONs must be able to meet the demands of a particular application without compromising on cellular toxicity. Our results showed that functionalization of SPIONs produced highly hydrophilic derivatives that are cytotoxic to NIH/3T3 cells. Nonetheless, this novel synthetic approach allows proceeding to further modifications to produce SPIONs with an improved design that can deliver their beneficial promises to the biomedical field."

24. Uncovering the molecular signature of bone marrow CD8+ T cells in myeloid malignancies

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Background: Myelodysplastic syndrome (MDS) is a diverse group of clonal hematopoietic progenitor cell malignancies, which is characterized by an elevated disposition of progression to difficult-to-treat secondary acute myeloid leukemia (AML). Even though MDS is a stem cell disorder, it has been associated with immune dysregulation. However, many unanswered questions remain about the role of adaptive immunity in the disease progression to this date.
Aim: In this study, we sought to investigate the immune landscape of bone marrow samples from patients with Chronic myelomonocytic leukemia (CMML), high-risk MDS (type RAEB-II) and AML.
Methods: To this goal, mass cytometry (CyTOF) was performed on isolated bone marrow mononuclear cells (BMMCs) and RNA-sequencing was utilized to define the transcriptome profile of bone marrow-derived CD8+ T cells.
Results: Immunophenotypic analysis highlighted differences between the three disease groups and identified the CD8+ T cell population, as the single immune cell type that differentiates the samples from patients with MDS from those with AML. RNA-sequencing analysis brought to light several molecular pathways, including IFN-a and IL-6/STAT3 signaling pathways, that distinguish the AML patients from the rest. Moreover, this analysis also revealed that the molecular signature of MDS patients responding to treatment (azacitidine) differs from that of AML patients, in contrast to non-responding MDS patients. In more detail, CD8+ T cells from patients that responded to treatment were found to overexpress genes related to autophagy, JAK/STAT and TNF signaling.
Conclusion: Herein, we show that the bone marrow immune microenvironment differs between MDS and AML. A particularly interesting finding was the significant similarity between the molecular profiles of CD8+ T lymphocytes of non-responding MDS patients and AML patients. Altogether, our study has provided useful insight into the key role of CD8+ T cells in myeloid malignancies.
Keywords: MDS, AML, CMML, CD8+ T cells, Mass cytometry, RNA-sequencing"

25. Development of an efficient system for seamless integration of transgenes in mouse Embryonic Stem Cells.

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Mammalian cell expression systems are common biotechnological tools with multiple practical uses. The traditional approach for developing recombinant cell lines is random insertion of the gene of interest (GOI) into the genome, followed by a selection for cells carrying the transgene. However, random integration is susceptible to unstable and variable expression and/or harmful alteration of the host genome1. On the other hand, site-specific integration by homologous recombination is more efficient and accurate but less efficient, time consuming and laborious.

The Hipp11 intergenic region is a “safe harbor” locus allowing homogeneous transgene expression without perturbing endogenous gene activity. Moreover, Hipp11 is not affected by neighboring regulatory elements allowing expression of transcripts by tissue specific promoters/enhancers2.

Using Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9 and φC31 integrase mediated irreversible recombination, we have developed a two-transfection step method for seamless integration of a gene expression cassette into the Hipp11 locus of mouse Embryonic Stem Cells (mESCs). This system can efficiently generate a fluorescent mESC line expressing high levels of tdTomato and can be potentially applied for the expression of a great variety of GOIs in different cell lines.

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26. McIdas affects ependymal cell differentiation in the mouse brain.

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Ependymal cells are specialized epithelial cells fundamental for the appropriate function of the subventricular zone, one of the main neurogenic niches in the adult mouse brain. Most ependymal cells, bear on their apical surface multiple cilia that beat unidirectionally, supporting cerebrospinal fluid flow. They derive from radial glial cells, committed towards this lineage during embryogenesis. A small percentage of ependymal cells bear two complex basal bodies that nucleate two motile cilia and are called biciliated ependymal cells. However, their developmental origin and their role have not been investigated.

Our lab research is focused on unraveling the molecular mechanism regulating the commitment and differentiation of neural progenitors towards ependymal cells in brain. Previous studies from our lab have indicated two members of Geminin superfamily, GemC1 and McIdas, as master regulators of the multiciliogenesis program.

In order to investigate the role of McIdas in ependymal cell differentiation, we generated mice that constitutively lack McIdas. McIdas knock-out mice are born in normal ratio but they display growth retardation, postnatal lethality and enlarged brain ventricles, which is a common characteristic of hydrocephalus. Through immunofluorescence in coronal brain sections we have shown that the expression of p73 and Foxj1 in McIdas KO mice is retained, indicating that progenitor cells are committed towards the ependymal lineage. However, when we examined whole mounts of lateral wall of brain lateral ventricles, we found that upon McIdas deletion, the differentiation of multiciliated cells is severely disrupted, as we could not detect multiciliated cells, while cells bearing one or two cilia have been detected.

In conclusion our data suggest that McIdas is not necessary for the commitment to the ependymal lineage, but affects the later steps of differentiation towards multiciliated ependymal cells and may be implicated in the biciliated ependymal cell formation."

27. McIdas in primary cilium formation

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

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

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

28. The effect of a SGLT2i on the development of neurons in primary cell cultures

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Aims:
Metabolic neuronal pathways and their effect on the central nervous system (CNS) play a key role in neuronal degeneration and death. Considerable number of drugs modulating these pathways have been tested so far. Empagliflozin, a drug initially used for glucose management of type 2 diabetes, comprises a promising novel therapeutic approach tested against neurodegenerative disorders.
Methods:
Primary neuronal cell cultures were developed from wild type Sprague Dawley rats and neurons were exposed to different concentrations of empagliflozin for 48 hours, during either their early development in the first week, or after two weeks in vitro. In parallel, fibroblast from NIH/3T3 cell line were cultured and exposed to the same and higher concentrations. Neuronal cells and fibroblasts' survival was evaluated by MTT assay and development of the dendritic tree was assessed under immunofluorescent microscopy after impregnation of neurons with CM-DiI. A morphometric comparative study was also performed with the ImageJ software and NeuronJ plug-in.
Results:
Empagliflozin at a concentration between 0,001μM and 8μM did not significantly alter the survival of the neurons and the development of their dendritic field. However, as expected, higher concentrations resulted in a reduction of total dendritic tree length, branching, and number of spines.

Comparatively, fibroblasts' survival did not alter even with higher concentrations of empagliflozin.

Conclusions:

Our results show that low and medium concentrations of empagliflozin on neurons do not exert any toxic effect. It is thus appropriate for further in vitro and in vivo testing as neuroprotective agent against degenerative disorders. However, higher concentrations of empagliflozin in the CNS may cause neurotoxicity, due to glucose deprivation on neurons. In the contrary NIH/3T3 cell line seems not to be toxically affected by higher concentrations perhaps mimicking mechanisms activated in other cells such as cardiac myocytes."

29. In vitro evaluation of the cytotoxic effect of extracts derived from natural products.

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Aims:
Natural products and their analogues exhibit prominent role in the treatment of a wide range of diseases including metabolic syndrome, inflammatory disorders, neurodegenerative diseases as well as different types of cancer. In the present study we tried to evaluate in vitro, the cytotoxic effect of curcumin and siderol extracts in fibroblast cells as well as in glioblastoma cells.

Methods:
First, we performed cell viability assay (MTT) on fibroblast (NIH) and human glioma cell cultures (U87). Both cell lines were treated with different concentrations of curcumin and siderol for 72 hours in order to estimate the effects of these extracts on the cell lines viability and proliferation as well as the IC50 value after 72h of treatment. To further delineate the effects of these two extracts on cell proliferation Crystal Violet staining of NIH and U87 cells was performed and photos using phase-contrast microscopy were taken at 72h, to evaluate if increasing concentrations of curcumin and siderol, induced changes in morphology and caused cell death on both cell lines.

Results:
Our study revealed inhibition of glioblastoma cell proliferation by both curcumin and siderol after 72h of treatment. Also increasing concentrations of the aforementioned agents led to morphological changes and induced cell death after the same period of time compared to control cell line. Curcumin and siderol seemed to have less toxic effect on fibroblast cells. The IC50 values for both agents on fibroblast cells, were 40μM for curcumin and between 60-70μM for siderol, in contrast the corresponding values for U87 cells were significant lower, namely 10μM for curcumin and 13μM for siderol.

Conclusions:

Conclusively, extracts derived from natural products such as curcumin and siderol, constitute potential therapeutic factors for the treatment of glioblastoma, the most aggressive and invasive primary brain tumor without being harmful for normal cells."

30. GemC1 is essential for normal hippocampal development in mice

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The hippocampus, located in the inner region of the mammalian temporal lobe, composes an intricate brain structure associated primarily with memory. The subgranular zone (SGZ), is a narrow layer of cells located in the dentate gyrus, whose main function involves the production of new neurons throughout adult life in most mammals, acting as a neurogenic niche. Many neurological diseases and disorders have been associated with changes in the neurogenesis that takes place in the SGZ, however the molecular mechanisms regulating these changes have yet to be fully elucidated.
Our study thus far suggests that GemC1, a member of the Geminin superfamily, regulates the transcriptional activation of p73 in vivo and direct interactions between GemC1 and p73 are pivotal for multiciliogenesis in mice. Concurrently, experiments on mouse models have shown that p73 is essential for normal hippocampal development and its replacement or depletion leads to severe hippocampal dysgenesis. Our objective is to investigate whether the absence of GemC1 affects the expression of the p73, as well as the rest members of the p53 family in the developing hippocampus, influencing both its structure and function. For this purpose, we have generated GemC1 knockout mice. Our study in embryonic mice, as of yet, suggests that GemC1 controls the expression of p53 and p73 during different developmental stages. Preliminary data also show that GemC1 deficiency leads to changes in various cell populations of the hippocampus, including astrocytes and neurons which are present in the SGZ, highlighting its importance on normal hippocampal development.
We presume that these findings will help uncover the molecular mechanisms underlying the hippocampal formation and assist in creating ideal models for the study hippocampus related diseases and disorders."

31. Reporting on the value of Artificial Intelligence in predicting the optimal embryo for transfer: A systematic review and meta-analysis

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In the era of Personalized Medicine and Precision Medicine, Artificial Intelligence (AI) constitutes a valuable tool that may enhance embryo selection for transfer in terms of precision and consistency. This study reports on the effectiveness of AI-based prediction models in predicting in vitro fertilization (IVF) outcomes through the evaluation of embryo's implantation potential. Following a systematic search of the literature in Pubmed/Medline, Embase, and Cochrane Central Library, 18 studies were identified as eligible for inclusion. Regarding live-birth, the Area Under the Curve (AUC) of the Summary Receiver Operating Characteristics (SROC) was 0.905, while the partial AUC (pAUC) was 0.755. The Observed:Expected (O:E) ratio was 1.12 (95%CI:0.26–2.37;95%PI:0.02-6.54). Regarding clinical pregnancy with fetal heartbeat the AUC of the SROC was 0.722, while the pAUC was 0.774. The O:E ratio was 0.77 (95%CI:0.54–1.05;95%PI:0.21-1.62). As far as the ploidy status is concerned, the AUC of the SROC was 0.751, while the pAUC was 0.585. The O:E ratio was 0.86 (95%CI: 0.42–1.27;95%PI: 0.03-1.83). This study demonstrates that the majority of the AI-based models could robustly predict IVF outcomes, including live birth, clinical pregnancy, clinical pregnancy with fetal heartbeat rate and embryo ploidy status. Although the innovative technology of AI prediction models seems to be revolutionary in the rapidly evolving field of IVF, our findings illustrate that the predictive capabilities of AI albeit accurate they are comparable to the human element, namely, the embryologists' evaluation. Nevertheless, further studies are needed in order to constitute AI as the gold standard in predicting embryos' potential and in turn IVF outcomes.

32. In vitro reprogramming of astroglial cells into ependyma

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The adult mammalian brain retains the ability to generate neurons through Neural Stem Cells residing in specialized niches, the subventricular zone (SVZ) niche of the lateral ventricles and the dentate gyrus of the hippocampus. Multiciliated ependymal cells are key components of the SVZ microenvironment. They line the walls of the lateral ventricles and carry multiple motile hair-like structures, called cilia on their apical surface. With their coordinated movement, these cilia control the cerebrospinal fluid (CSF) flow through the ventricular system and are crucial for the proper brain development and function. Importantly, cilia malfunction can lead to various brain disorders, like hydrocephalus.

Previous work from our lab has highlighted the two geminin family members, GemC1 and McIdas, as critical regulators of multiciliate cell fate acquisition and differentiation in the brain. Up to date, GemC1 has been proven to be the most upstream molecule in the hierarchy of the multiciliate differentiation program. Our laboratory has shown that GemC1 upregulates the expression of important transcription factors of multiciliogenesis, such as McIdas, Foxj1 and p73, thus governing the generation of multiciliated cells. Of note, GemC1-deficient mice are devoid of multiciliated ependymal cells and develop hydrocephalus.

Astrocytes, which are one of the most abundant types of neural cells in the brain, play important role in neurodegeneration and exhibit remarkable plasticity in vivo. Upon brain damage, including hydrocephalus occurrence astrocytes become activated to reestablish homeostasis and the lost functions of ependymal cells. Here we show that GemC1 and McIdas are capable of inducing astrocytes differentiation towards the ependymal cell lineage in vitro. Our study shows that both GemC1 and McIdas can downregulate the astrocytic characteristics and promote the generation of functional ependymal cells.

This type of approach could be used in the future as novel therapeutic intervention for hydrocephalus treatment."

33. The impact of physical activity on semen parameters in healthy individuals: A prospective observational single-center study

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Physical activity seems to be an integral part of daily lives of people in the modern era. The literature regarding the effect of physical activity on semen parameters has not drawn a proper conclusion as far as their association is concerned. The majority of these studies have focused on athletes, men of excessive physical activity, men with sedentary life or individuals presenting with infertility. This study evaluates the impact of exercise on semen parameters in an otherwise healthy population. The participants comprised of 223 healthy individuals with a free medical history and no previous diagnosis of male infertility. They were interviewed according to the Global Physical Activity Questionnaire (GPAQ) and then, divided into three groups, namely, the low, moderate and high physical activity groups according to their Metabolic Equivalents (METs) profile. Semen analysis was performed according to the fifth edition of WHO laboratory manual. The findings showed 35% less probability of abnormal semen quality in men with moderate physical activity compared with men with low physical activity (RR: 0.65 95%CI: 0.52-0.81). Further to this the moderate physical activity group presented with higher total motility (43.08 ± 18.34 vs 36.43 ± 16.01; p-value = 0.01) and higher progressive motility (31.86 ± 17.69 vs 25.75 ± 16.50; p-value = 0.04) when compared to the low physical activity group. No statistically significant difference was observed regarding sperm concentration or normal morphology forms. Nevertheless, individuals in the higher physical group demonstrated marginally insignificant impaired semen parameters as opposed to men with moderate training. Ultimately, on the account of the existing evidence, moderate physical activity seems to be correlated with optimal semen quality and parameters. However, more studies are needed to confirm this association.

34. Dysregulation of the endometrial interleukin network during the peri-implantation period could lead to recurrent implantation failure: A comprehensive review of the literature

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Recurrent implantation failure (RIF) is a multifactorial condition affecting 10–15% of in vitro fertilization (IVF) couples. Data suggest that functional dysregulation of the endometrial immune system constitutes one of the main pathophysiological mechanisms leading to RIF. The aim of this article is to provide a thorough presentation and evaluation of the role of interleukins (ILs) in the pathogenesis of RIF. A comprehensive review of the literature was performed in PubMed/Medline, Embase, and Cochrane Central databases up to November 2021. The only prerequisite for a study to be considered eligible for inclusion was the clear description of the results and the methodology employed. Literature analysis revealed that during implantation, several classes of ILs are secreted by epithelial and stromal endometrial cells. These ILs create a perplexing network that orchestrates both proliferation and maturation of uterine natural killer cells, controls the function of regulatory T and B cells inhibiting the secretion of antifetal antibodies, and supports trophoblast invasion and decidua formation. Alterations in expression patterns of ILs in the maternal–fetal interface negatively affect numerous biological processes required for proper embryo implantation and pregnancy establishment. These include decidualization, immunological acceptance of the semi-allogenic blastocyst, embryo–maternal communication, embryo attachment to the endometrium, trophoblast cell invasion, spiral artery remodeling, embryo development, and several others. Moreover, data highlight the significant role of ILs in establishing a balanced Th1/Th2/Th17 and Treg microenvironment during the peri-implantation period, which in turn is required for implantation and proper development of the early-stage embryo. This review further proposes a mapping of future research on how to move forward from mere associations to robust molecular data that will allow an accurate profiling of ILs in turn enabling evidence-based consultancy and decision making when addressing RIF patients.

35. Molecular Drivers of Developmental Arrest in the Human Preimplantation Embryo: A Systematic Review and Critical Analysis Leading to Mapping Future Research

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Developmental arrest of the preimplantation embryo is a multifactorial phenomenon, occurring on different developmental stages and characterized by a failure of cellular division for at least 24 hours. The present systematic review aims to investigate all the molecular drivers entailed in embryo arrest, focusing on embryonic and parental factors. A systematic search in PubMed/Medline, Embase and Cochrane-Central-Database was performed in January 2021 and a total of 76 studies were included. The embryonic factors indicating association with developmental arrest included gene variations, mitochondrial DNA copy number, methylation patterns, chromosomal abnormalities, metabolic profile and morphological features. Parental factors included gene variation, protein expression levels and infertility etiology along with their respective genetic background that may be indirectly associated with arrest. Indeed, considering the role of maternal factors, it becomes clear that advanced maternal age is identified as a dominant cause of embryo arrest, as it has been associated with embryo morphology and chromosomal abnormalities. However, it is apparent that genetic factors exert the greatest influence on embryonic development, as they impact the core identity of the embryo and cannot be bypassed. An important finding emerging through an in-depth critical analysis demonstrated that genetic sources of developmental arrest analyzed from the perspective of parental infertility etiology and the embryo itself, share common ground. This study is a distinctive contribution to literature that for the first time presents all the wealth and volume of data on the subject of developmental arrest giving an all-inclusive report on the molecular drivers leading to this phenomenon. Information provided herein might support the development of novel approaches to increase the possibilities for a successful in vitro fertilization (IVF) outcome. Successful

identification of the drivers leading to developmental arrest, will increase knowledge on one of the most complicated matters in assisted reproduction and one that is currently underexplored.

36. The effect of oocyte vitrification accounting for both open and closed systems on embryo developmental arrest rate. A systematic review and network meta-analysis

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Oocyte vitrification constitutes the gold standard method of cryopreservation. The universal employment of oocyte vitrification warrants further investigation of its impact on embryo developmental competency. The current meta-analysis aims to study the developmental arrest rates of embryos deriving from vitrified oocytes, and possible differences between open and closed vitrification systems (VS). A systematic search of the literature was conducted, resulting to 17 prospective studies being included. The population of included studies consists of vitrified or fresh oocytes, subjected to ICSI. A network meta-analysis was performed comparing the type of VS employed and fresh oocytes. The eligible studies presented with high heterogeneity(I2=81%). Statistically higher embryo developmental arrest rate per MII oocyte vitrified, employing either the open or closed VS compared to fresh oocytes observed (open-systems: RR:1.16;95%CI:1.07-1.26; closed-systems: RR:1.19;95%CI:1.06-1.34). No statistically significant difference was observed between open and close VCs (RR:0.99;95%CI:0.89-1.10). Subgroup analysis was performed according to the developmental stage of embryo arrest. A statistically significant difference on developmental arrest of embryos prior to the cleavage stage was identified when vitrifying (open-systems: RR:1.44;95%CI:1.18-1.77; closed-systems: RR:1.51;95%CI:1.12-2.04; 8 studies). However, no statistically significant difference on developmental arrest of embryos prior to the blastocyst stage was observed (open-systems: RR:1.06;95%CI:0.98-1.15; closed-systems: RR:1.10;95%CI:0.98-1.24; 9 studies). Fertilization rate was significantly lower for vitrified oocytes compared to fresh (open-systems: RR:0.86; 95%CI:0.79-0.93; closed-systems: RR:0.81 95%CI:0.72-0.92), while no statistically significant difference was observed between the two VSs (open vs closed: RR:1.04;95%CI:0.93-1.16). When comparing developmental arrest rate per 2PN zygote no statistically significant difference was detected between vitrification versus fresh (open-systems: RR:1.01;95%CI:0.87-1.17; closed-systems: RR:0.98;95%CI:0.78-1.22), or between the two VSs (open vs closed: RR:1.03;95%CI:0.82-1.30). To conclude, oocyte vitrification results to

higher developmental arrest rates per oocyte vitrified but not per 2PN zygote compared to fresh. The two VS systems do not seem to present with statistically significant different outcomes.

37. MicroRNA profiling may be a promising tool for unveiling idiopathic non-obstructive azoospermia pathogenesis: A systematic review and in-silico meta-analysis of the affected pathways

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Despite the advances in the field of reproductive medicine the exact infertility aetiology remains unidentified regarding 30-40% of infertile men. This fact highlights the need for more accurate and sensitive diagnostic tools, especially regarding the most severe cases, such as idiopathic non-obstructive azoospermia (NOA). This study aims to investigate possible associations between microRNAs in seminal plasma and microRNAs in testicular tissue samples obtained from idiopathic NOA patients. A systematic review was performed in PubMed/Medline and Embase. Only original retrospective or prospective human studies were included. The studied population consisted of idiopathic NOA patients, while the control group consisted of fertile men or men with normal semen analysis. Original data on altered microRNAs were analyzed aiming to underline differences between microRNA expression profiles in seminal plasma and testicular tissue samples. Following this, in-silico analysis was performed to detect commonly affected gene expression pathways, employing a combination of bioinformatic tools, namely the DIANA-TarBase, microT-CDS, the GTEx repository and the KEGG database. Statistical analysis was performed using the R-package-limma. Five studies were considered eligible, including 382 NOA cases and 412 controls. Two studies co-evaluated the profile of microRNAs in both seminal plasma and testicular tissue samples, one study evaluated only testicular tissue and the other two only seminal plasma. Data extraction revealed a total of 14 differentially expressed microRNAs between NOA patients and controls. Interestingly, in-silico analysis revealed 34 statistically significant dysregulated gene pathways, regarding both seminal plasma and testicular tissue samples, indicating that idiopathic NOA patients are sharing several common altered molecular mechanisms. In summary, this study

identified common alterations in microRNA profiles and gene expression patterns between seminal plasma and testicular tissue samples in NOA patients. These findings indicate that microRNA profiling in seminal plasma could indeed be introduced as a powerful non-invasive tool towards better understanding and diagnosing idiopathic NOA.

38. Controlling centriole numbers in cells

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Centriole numbers in cells are tightly controlled to ensure bipolar spindle assembly. Aberrations in centriole numbers lead to genomic instability and cancer1. The tight control of the expression levels and the interactions of PLK4, STIL and SAS6 have been proposed as predominant mechanisms for centriole numbers, to avoid reduplication in the same cell cycle. However, our understanding of the fundamental principles that govern this process is still poor. Here, we characterized McIdas as a novel protein that contributes to a normal centriole cycle. McIdas along with Geminin and GemC1 constitute the Geminin superfamily with significant roles in cell cycle2,3 and in centriole amplification during multiciliogenesis4-8.

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

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

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39. Study of signaling mechanisms involved in dithiothreitol- and tunicamycin-induced endoplasmic reticulum stress in H9c2 cells

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Under physiological(1) as well as pathophysiological(2) conditions proper protein folding may be compromised, resulting in accumulation of aggregated proteins in the endoplasmic reticulum (ER) lumen, thus activating ER stress. ER stress is implicated in a number of pathologies including cardiovascular disorders(3). Therefore, elucidating the signal transduction pathways mediating the responses elicited is of great importance.

Accordingly, the present study aimed at unraveling the cellular responses induced by dithiothreitol (DTT-4 mM) and tunicamycin (TN-0.1 μg/ml) in H9c2 cardiomyoblasts. Cell viability was assessed by MTT assay and cell lysates were used for Western blot analyses.

Treatment with DTT as well as TN caused a dose-dependent decrease in H9c2 cellular viability. Of note, the cell death conferred was linked to apoptosis only in the case of DTT, evidenced by detection of PARP fragmentation. Treatment with TN did not stimulate PARP proteolysis.

Since phosphorylation of eIF2α constitutes a fundamental step in the ER stress response, we next examined its time-dependent profile under the conditions investigated. Interestingly, 4 mM DTT caused a rapid increase in the phosphorylation levels of eIF2α, maximized after 15 minutes. On the other hand, treatment with 0.1 μg/ml TN was found to induce a biphasic and prolonged phosphorylation pattern.

With accumulating data highlighting the interrelation between ER stress and autophagy, induction of the latter was subsequently examined. Protein levels of Beclin-1 were maximized as early as 30 min after DTT treatment, while the response was more delayed after treatment with TN.

Overall, compared to TN, DTT was observed to cause a more robust ER stress response, in parallel with autophagy and apoptosis. Further studies are however required so as to probe into the effectors mediating these responses and the adaptive mechanisms initiated.

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40. The effects of caloric restriction and exercise in steroid production in obesity and cardiometabolic disease

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Background and Aims: Obesity-associated cardiometabolic disease consists a major health problem. Elevated levels of adrenal steroids (cortisol and aldosterone) are present in obesity contributing to hypertension and cardiometabolic disease. Their production is controlled by the adrenocorticotropic hormone (ACTH) which is produced by expression of the proopiomelanocortin (POMC) gene. Herein, we sought to highlight the control of steroidogenesis during development of cardiometabolic disease and investigate its possible reversal through dietary interventions and exercise. Methods: Male C57Bl6 mice were subjected to 5 different diet and exercise regimens for 21 weeks: normal fat diet (ND) feeding, high fat diet (HFD) feeding, HFD feeding followed by switch to ND for the last 9 weeks, HFD feeding along with aerobic exercise for the last 9 weeks, HFD feeding followed by switch to ND along with aerobic exercise for the last 9 weeks. For the cardiovascular assessment, echocardiography and blood pressure measurement were conducted. Following the experiments, several biochemical and steroids measurements in blood and excised tissues were carried out for phenotypic characterization of the mice. The gene expression responsible for steroidogenesis was evaluated by qPCR. Results: Blood pressure determination along with tissue weights display obesity development, as well as its reversal through our interventions. When compared individually, dietary intervention seems more effective than exercise. Aldosterone and corticosterone levels in serum and adrenal tissue are elevated in HFD-fed mice. Expression analysis of the POMC gene validates the elevated levels of ACTH in blood circulation. Conclusion: Controlling steroidogenesis is crucial for the development of obesity and the establishment of its cardiometabolic effects, as well as their reversal through dietary and/or exercise interventions. The ACTH levels along with its gene expression suggest its central role in the regulation of steroidogenesis. Future work could demonstrate DNA methylation of the POMC gene as a possible epigenetic director of this process.

41. Gastroesophagical reflux as a predictive factor of VAP in mechanically ventilated patients.

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Mechanical ventilation is used for replacing the breathing of intubated patients. However, there are some complications observed in mechanically ventilated patients (MV), including gastroesophagical reflux (GOR) which can lead to the invasion of microbes from the digestive system to the respiratory tract. MV patients often develop Ventilator Associated Pneumonia (VAP), a main factor of bad prognosis that could lead to a 70% increase in ICU mortality. The present study aimed to examine the use of GOR as a predictive factor of VAP manifestation.

67 patients, age 63,16 ± 16,41 years old, admitted in the ICU of the University Hospital of Larissa (2018-2020) were enrolled in the study. In all patients, intra-abdominal pressure (IAP) was recorded and a bronchial secretion sample was received from the aspiration tube. Secretions were mechanically dissolved and total protein and albumin were chromatometrically measured, using the Bradford and the Bromocrezol-Green method, respectively. Furthermore, pepsin concentration was evaluated using ELISA.

Patients had a 17,42 ± 6,989 mean APACHE II score, with 10,11 ± 9 days of MV. 35.82% of the patients developed VAP. Albumin was the 80% of total protein concentration, while total protein was positively corelated (r=0.8, Pearson) to the pepsin concentration, showing that total protein could be representative of pepsin presence. pH values were irrelevant of the presence of pepsin in secretions, showing that GOR does not affect the pH of bronchial secretions. In addition, IAP measurements were positively correlated to the concentration of pepsin in bronchial secretions (r=0.7, Pearson), showing that when IAP is elevated GOR is more likely to happen.

Conclusively, the elevation of IAP leads to GOR that increases the possibility of VAP manifestation in MV patients. The measurement of IAP could represent a good prognostic tool for GOR and furthermore, the manifestation of GOR could be a predictive factor of VAP."

42. Tiotropium effect on the proliferation and the phenotype of Airway Smooth Muscle Cells

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Chronic lung diseases, such as asthma or chronic obstructive pulmonary disease (COPD), are characterized, among others, by bronchospasm and structural modifications of the airway wall. Such changes include epithelium damage, thickening of the basal membrane, increase in vessel number, as well as hyperplasia and/or hypertrophy of the airway smooth muscle cells (ASMCs). Tiotropium, a classic M2/M3 muscarinic antagonist, is widely used as bronchodilator, mainly in COPD, but also in asthma treatment. We studied the effect of tiotropium on ASMC proliferation and phenotype in rabbit tracheal primary cell and human bronchial smooth muscle cell cultures.

Cell proliferation and the signaling pathways involved were estimated with Trypan Blue staining and the use of PI3K and MAP kinases pathways' inhibitors while the tiotropium effect on cell phenotype was assessed with the immunofluorescent localization of α-actin and heavy myosin chain (MHC).

Our results show that in the absence of any muscarinic agonist, low concentrations (1-10nM) of tiotropium induce ASMC proliferation in a dose-response manner, whereas in higher concentrations (20nM or 30nM) no effect was observed. Similarly, in human ASMCs, tiotropium (5nM or 10nM) had a mitogenic effect. In rabbit SMCs, the effect of tiotropium is achieved through the activation of the PI3K and MAP kinases signaling pathways, since treatment with the pathways' antagonists, LY294002 and PD98059 respectively, abolish tiotropium effect. Indirect immunofluorescence experiments revealed that incubation with 5nM tiotropium, for 48 hours, does not alter α-actin or MHC expression in the ASMCs, an effect similar to that of 10% FBS.

In conclusion, in the absence of agonists, tiotropium (1-10nM) has a mitogenic effect on human and rabbit ASMCs via activation of PI3K and MAP kinases' signaling pathways without altering cell phenotype."

43. Azithromycin induces autophagy in Vascular Smooth Muscle Cells.

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Respiratory and vascular inflammation lead to airway/vessel wall remodeling including hyperplasia and/or hypertrophy of smooth muscle cells. Azithromycin, a macrolide antibiotic, is used in low-dose, long-term treatment of chronic respiratory and cardiovascular inflammatory diseases. The aim of the present study was to investigate the effect of azithromycin on vessels, using both rabbit aortic SMC primary cultures (cellular level) and rabbit aortic rings (tissue level).

Cells and tissues were incubated with azithromycin (1-10μM) in the presence and absence of 10% FBS for 24-72 h. In cell cultures, the effect of azithromycin on autophagy was observed microscopically, in the presence or absence of the autophagy inhibitor 3methyladenine (3MA, 5mM). Cell viability was evaluated with Trypan blue staining. In aortic rings, the azithromycin (10μm) effect was studied after tissue sectioning and with the use of Cresyl Violet and Toluidine Blue stains as well as immunofluorescent localization of the autophagy markers Beclin and LC3.

In vascular smooth muscle cell cultures, cell incubation for 24-72 h with azithromycin (1-10μM), in the presence or absence of 10% FBS, reduces cell number and induces the appearance of autophagic vacuoles. This effect was reversed by either azithromycin exclusion from the incubation medium or in the presence of 3MA (5mM). Furthermore, incubation of aortic rings for 24h with azithromycin (0.1 or 10μM) increased Beclin and LC3 immunoreactivity and promoted a wavy smooth muscle appearance.

In conclusion azithromycin, induces autophagy in vascular smooth muscles both in the cellular and the tissue level."

44. Poor responder patients present with increased Prokineticin-1 follicular fluid levels indicating abnormal ovarian vascularization: A prospective observational study

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Prokineticin-1 (PROK1) is an angiogenic factor with pleiotropic properties. Recently published studies indicate that PROK1 is associated with ovarian function and oocyte competence. However, limited data are available on associating PROK1 with poor ovarian response (POR). The aim of the present prospective observational study was to investigate possible associations between PROK1 follicular fluid (FF) levels in POR cases, with stimulation outcome, as well as with FF levels of other factors related to ovarian functionality, namely VEGF, BMP-15 and PEDF. A total of 64 patients undergoing IVF treatment were enrolled. The study group comprised of 32 POR patients defined according to the Bologna criteria. The control group consisted of 32 normal responder women. Participants received the standard short GnRH-antagonist protocol. The FF samples were collected as part of the oocyte retrieval process, then centrifuged and stored at -80 °C till analysis. Proteins FF levels were evaluated via ELISA. Significantly higher PROK1 (P-value<0.0001), VEGF (P-value=0.006) and lower BMP-15 (P-value=0.001) levels were recorded in the POR group. No difference was observed regarding PEDF levels. However, the POR group presented with lower PEDF/VEGF ratio (P-value=0.02), indicating a reduced antioxidant capacity. PROK1 levels were negatively correlated with AMH (P-value=0.04), number of oocytes retrieved (P-value=0.001) and number of MII oocytes (P-value=0.005). PROK1 levels, with a cut-off value of 2854.25 pg/ml, were able to predict ovarian response status with an area under the curve at 0.64. Sensitivity was 0.55, specificity was 0.88, and accuracy was 0.71. The positive and negative predictive values were 81.82% and 66.67%, respectively. In summary, data presented herein indicate that PROK1 FF levels are strongly associated with POR. Considering that FF-PROK1 presents with a similar profile with FF-VEGF, we can form the hypothesis that the compromised angiogenesis observed in POR patients leads to PROK1 and VEGF increase via a negative-feedback-loop.

45. Hepcidin as a sensitive and treatment-responsive acute-phase marker in patients with bacteremia: a pilot study

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Hepcidin plays a pivotal role in iron metabolism by inhibiting both intestinal iron absorption and iron release from iron stores. Iron overload up-regulates while iron depletion down-regulates hepcidin synthesis in order to maintain plasma iron concentration. Inflammatory conditions also up-regulate hepcidin synthesis, which is hypothesized to serve as an antimicrobial defense mechanism by reducing the availability of circulating iron to the invading microbes. To test this hypothesis in human patients, serum hepcidin concentration [median(IQR)ng/ml] was determined by ELISA in healthy blood donors (n=60) and patients hospitalized because of bacteremia (n=50), upon their admission (day 0) and after seven days of antibiotic treatment (day 7). Serum hepcidin levels were tested for correlation to serum ferritin in order to check the physiological operation of the iron-hepcidin axis. Serum hepcidin of healthy subjects was 18(21)ng/ml and positively correlated to ferritin ($r=0.62$, $p<0.0001$). Serum hepcidin was found significantly increased in patients with bacteremia both at day 0 [123(158)ng/ml] and at day 7 [67(68)ng/ml] compared to healthy controls ($p<0.0001$ for both). However, there was significant reduction of serum hepcidin after 7-day antibiotic treatment of the patients ($p<0.0001$). In contrast, serum ferritin, which was also high in the patients, was not significantly affected by the antibiotic treatment ($p=0.6571$), resulting to significant positive correlation between serum hepcidin and ferritin values at day 7 ($r=0.74$, $p<0.0001$) but not significant correlation ($r=0.29$, $p=0.0702$) at day 0. These data confirm the inflammatory stimulation of hepcidin synthesis in human subjects and support the hypothesis that hepcidin also contributes to host anti-microbial defense. In addition, hepcidin appears to be a more sensitive and treatment-responsive acute-phase marker than ferritin in patients with bacteremia.

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46. PRENATAL ALCOHOL EXPOSURE AFFECTS NEURONAL SYNCHRONIZATION MECHANISMS, VIA K+ CONDUCTANCES AND GAP JUNCTIONS, IN DEVELOPING AND ADULT INTERMEDIATE HIPPOCAMPUS IN VITRO

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Fetal Alcohol Syndrome (FAS) results from fetal exposure to alcohol and correlates with several structural and functional CNS disorders, increased epileptic seizure incidence being one of them, cognitive problems another. However, the neuronal mechanisms leading there are not known. We investigated the effects of mild Prenatal Alcohol Exposure (PAE) throughout pregnancy in the characteristics of synchronous interictal epileptiform discharges (IEDs) recorded from the CA1 area of intermediate* (*chosen because of its importance in memory and learning) hippocampal slices.
Exposure of female SD rats to Alcohol and PAE-offspring generation was done as reported earlier[1]. Spontaneous (IEDs) were pharmacologically induced in vitro in hippocampal slices from PAE and age-matched control (N) rats (Young, 21-35PND; Adult, >90PND) and their features were compared following specific challenges.
IED frequency was lower in Mg2+-free ACSF, but higher in 4-aminopyridine (4-AP, 50μM) in PAE vs N Young slices ($p<0.0001$), suggesting differences in excitatory mechanisms (NMDA-Rs; K+ conductance block). Increasing extracellular K+ to 7mM in standard ACSF induced higher frequency spontaneous IEDs in PAE-Young slices (vs N, $p=0.008$), an effect reversed in A-slices. Moreover, extracellular K+ increase (to 7mM) in Mg2+-free ACSF, enhanced IED frequency significantly more in Young PAE slices (vs N) but not in Adult, suggesting the alleviation of some differences by aging. The Kir channel blocker BaCl2 (2mM) increased IED frequency significantly in all N but not PAE slices (Y&A), suggesting a long-term reduction of this type of K+ conductance there. The gap junction blocker carbenoxolone (300μM) decreased IED frequency in Young-PAE slices significantly more than in N ($p=0.01$), having no effect in A slices.
In conclusion, mild alcohol exposure throughout pregnancy provokes changes in hippocampal gap junctions and K+ conductances, likely affecting hippocampal synchronization modalities and therefore memory processing and seizure threshold.

[1] Evangelaki & Psarropoulou, Int J Dev Biol (2021)."

SELECTED FOR ORAL PRESENTATION

47. Integrated proteomic and lipidomic analysis of High-Fructose Corn Syrup-induced NAFLD in Obesity

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Background: High Fructose Corn Syrup (HFCS) is a sweetener rich in sucrose and fructose widely used in western diets, and its consumption has been associated with the emergence and progression of Non-Alcoholic Fatty Liver Disease (NAFLD). The additive impact of fructose on NAFLD phenotype has been proved in numerous studies, however, the exact mechanisms by which fructose influences hepatic metabolism – especially in the form of HFCS – are not known. Besides, a metabolomic characterization of a realistic NAFLD model that simulates synchronous malnutrition is missing. Aim: Our study focused on characterizing the additive impact of HFCS on NAFLD -related hepatic steatosis, specifically aiming to unravel the nature of mechanisms that could govern the HFCS -induced exaggeration of NAFLD in obesity. Methods: C57Bl6 male mice were fed a normal fat diet (ND) or a high fat diet (HFD), or a HFCS55 supplemented HFD (HFD-HFCS), for 20 weeks. Hepatic tissues were examined histologically, and starved serums were biochemically analyzed. Proteomic and lipidomic characterizations were conducted in hepatic tissues and the respective results were analyzed with Ingenuity Pathway Analysis software, to explore the biological background of the deteriorated HFD-HFCS phenotype. These results were further investigated with enzymatic assays, which focused on specific mitochondrial functions. Results: HFD and HFD-HFCS mice displayed comparable obesity, although HFD-HFCS mice showed greater aggravation of hepatic steatosis. In the HFD-HFCS hepatic proteome we observed increase in all de novo lipogenesis (DNL) enzymes, compared to HFD group. Computational analysis of metabolomic data suggested mitochondrial oxidative networks, such as OXPHOS and TCA cycle, as candidate mediators of steatosis deterioration in HFD-HFCS livers. Conclusion: The presence of extensive hepatic steatosis in HFD-HFCS mice as compared to the HFD ones, is attributed to increased DNL, and is probably orchestrated by mitochondrial dysfunction that implicates TCA cycle.

48. Lipofuscin follicular fluid levels indicate extensively increased cellular senescence in poor responder patients: A prospective observational study

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Lipofuscin levels have been associated with low oocyte competence and age-related infertility. However, hitherto no data has been published indicating the value of lipofuscin follicular fluid (FF) levels as a biomarker towards accurately predicting ovarian reserve and response. The aim of the present prospective observational study was to examine whether lipofuscin FF levels could be used as a sensitive biomarker for predicting poor ovarian response (POR). Thirty-two patients undergoing IVF were included, 16 diagnosed as POR according to the Bologna criteria and 16 normal responders. Following the oocyte retrieval, lipid and protein parts of lipofuscin were isolated from FF samples, stained with GL13 and labelled with an anti-biotin HRP conjugated antibody. Luminescence was measured with signal intensity indicating lipofuscin concentration. Poor responders presented with a statistically significant seven-fold higher lipofuscin FF levels in comparison to the normal responders (869.21 ± 501.87 vs 146.6 ± 107.64 RLU; P-value <0.0001). Lipofuscin levels were negatively correlated with AFC (P-value <0.0001), AMH levels (P-value =0.0002), number of oocytes retrieved (P-value =0.001), number of mature metaphase II (MII) oocytes obtained (P-value =0.0008), number of normally fertilized zygotes (P-value =0.003), number of cleavage stage embryos (P-value =0.005) and number of blastocyst stage embryos (P-value =0.02). Lipofuscin FF levels, with a cut-off value at 294, successfully predicted the ovarian stimulation response status with an AUC at 0.96. The sensitivity was 0.875, the specificity was 0.938 and the accuracy was 0.906. The positive predictive value was 88.24% and the negative predictive value was 93.33%. Data presented herein indicates, for the first time in literature, that lipofuscin FF levels measured via the GL13 method may be a promising and sensitive tool for predicting POR and stimulation outcome. Lipofuscin could further serve as a valuable novel biomarker indicating ovarian senescence, ovarian reserve status and oocyte competence.

49. Enhancement of the anticancer potential of luteolin after enzymatic modification with Thermomyces lanuginosus lipase

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Background: Luteolin is a common flavonoid that exists in many types of plants including fruits, vegetables, and medicinal herbs. This compound has multiple biological effects such as anticancer, antioxidant and anti-inflammation. Like many flavonoids, luteolin presents several hydroxyl groups, which limits their applications in some fields, due to their low solubility in lipophilic systems. To avoid that, luteolin can be acylated by lipase, using vinyl ester as acyl-donor. The enzymatic method is more selective and occurs in mild reaction conditions, in comparison with classic chemical methods.

Aim: The cytotoxic evaluation of the luteolin derived after enzymatic modification with Thermomyces lanuginosus lipase (TLL) and its comparison with the corresponding action of the non-modified compound.

Methods: The cytotoxic effect of the modified luteolin and luteolin was studied in vitro against cancer (leiomyosarcoma, LMS cells) and normal cells (fibroblasts, NIH/3T3 cells). The techniques that were used were cytotoxicity assays (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide-MTT assay and clonogenic assay) and flow cytometry analysis (2',7'-dichlorofluorescin-diacetate staining) for the detection of reactive oxygen species (ROS).

Results: Both standard and enzymatic mono-acetylated luteolin (3´-O-acetyl luteolin and 4´-O-acetyl luteolin) showed dose- and time-dependent cytotoxic effects against cancer cells. Also, the toxicity of modified luteolin was significantly lower in normal fibroblasts than that of the standard compound. Both compounds caused irreversible damage to cancer cells by inhibiting their proliferation. Finally, modified luteolin presented a greater ability to scavenge intracellular ROS and was found to induce apoptosis in leiomyosarcoma cells.

Conclusion: Enzymatic modification of luteolin with TLL enhanced the biological effects of luteolin. Further molecular experiments will unfold more details about the compound’s mechanism of action.

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50. Calcium homeostasis in the regulation of neuronal mitophagy

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Mitochondria are essential for energy production and have vital roles in calcium signaling and storage, metabolite synthesis and apoptosis, among others, in eukaryotic cells. Neuronal cells are particularly dependent on proper mitochondrial function. Thus, maintenance of neuronal homeostasis necessitates a tight regulation of mitochondrial biogenesis, as well as, the elimination of damaged or superfluous mitochondria. Mitochondrial impairment has been implicated in several age-related neurodegenerative diseases. Mitophagy is a selective type of autophagy mediating elimination of damaged mitochondria, and the major degradation pathway, by which cells regulate mitochondrial number in response to metabolic state. However, little is known about the effects of mitophagy deficiency in neuronal physiology. To address this question, we developed two composite, in vivo imaging approaches to monitor mitophagy in neurons. Neuronal mitophagy is induced in response to oxidative stress. Mitochondrial dysfunction leads to transportation of axonal mitochondria towards the neuronal cell body, in calcium- and an AMPK-dependent manner. Autophagy deficiency increases mitochondrial number in neurons of age-matched nematodes and abolishes mitochondrial axonal transport upon stress. Additionally, impairment of mitophagy results in enhanced cell death in C. elegans models of neurodegeneration. Our results indicate that mitophagy contributes to preserve mitochondrial homeostasis and neuronal health.

51. Application of chitosan nanoparticles to enhance the biological activity of luteolin

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Background Luteolin (3′,4′,5,7-tetrahydroxyflavone), belongs to a group of naturally occurring compounds called flavonoids that are found widely in the plant kingdom. Luteolin exhibits a wide range of biological properties including antioxidant, anti-inflammation, and anticancer effects. However, its poor bioavailability and hydrophobicity, restrict clinical application. Chitosan nanoparticles have been widely used to in pharmaceutical field to improve bioavailability and enhance absorption of bioactive compounds.

Aim To investigate the effects of luteolin-loaded chitosan nanoparticles on normal cells.

Methods The NIH/3T3 cell line (murine fibroblast) was used. The cytotoxic activity of luteolin and luteolin-loaded chitosan nanoparticles (50% w/w) was assessed utilizing 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and clonogenic assays and reactive oxygen species (ROS) were quantified with flow cytometry (2',7'-dichlorofluorescin-diacetate staining).

Results Encapsulation of luteolin to chitosan nanoparticles enhanced its cytotoxic activity against NIH/3T3 cells. Luteolin nanoparticles significantly suppressed the clonogenic potential of NIH/3T3 cells to a greater extent than luteolin. On the other hand, chitosan coating boosted the antioxidant potential of luteolin and its ability to scavenge H2O2-induced ROS (almost a 4-fold increase).

Conclusion Luteolin-loaded chitosan nanoparticles exerted higher toxicity than luteolin likely due to amplified uptake by the NIH/3T3 cells. Considering that cellular uptake of chitosan nanoparticles depends on their size and type of cells, modifying luteolin:chitosan ratio and applying them to several cell lines (cancer and normal) will provide more evidence to support their use as potential therapeutic agents.

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52. Promising graphene-based nanomaterials for biomedical applications: an in vitro toxicity assessment study

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Background: Due to their unique physicochemical properties, the use of graphene-based nanomaterials in biomedical applications has attracted great interest over the last decade. Nonetheless, evaluation of the safety and biocompatibility of a nanomaterial is a crucial prerequisite before its use in biomedical applications. Recently, several green exfoliation methods have emerged as more economical and environmentally friendly approaches for producing graphene from graphite.
Aim:To study the toxicity and biocompatibility of nitrogen-doped graphene (N-graphene) that has been synthesized via both chemical and green procedures.
Methods: In vitro assays in HaCaT (keratinocytes) and NIH/3T3 (fibroblasts) cells were performed. Cytotoxicity was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and the clonogenic assay. The redox stage of the cells was also examined through the detection of reactive oxygen species (ROS) generation using 2',7'-dichlorofluorescin-diacetate (DCF-DA) and flow cytometry.
Results: Chemically exfoliated N-graphene was more toxic than green exfoliated N-graphene at doses lower than 10 μg/ml in both cell lines. Greater reduction (70%) in cell viability was seen in HaCaT cells after incubation with 200 μg/ml of chemically exfoliated N-graphene for 48 hours. None of the two nanomaterials generated intracellular ROS. NIH/3T3 and HaCaT cells exposed to chemically exfoliated N-graphene were able to form colonies at lower rates than to green exfoliated N-graphene (90%).
Conclusion: Green exfoliation of graphite results in the production of a less cytotoxic N-graphene compared with the chemically exfoliated. Further

research on the activation of molecular pathways of inflammation by green N-graphene could prove its value for use in biomedical applications (i.e., biosensing, drug delivery etc.).

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53. Monitoring mouse hippocampal spontaneous activity using voltage sensitive dyes

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Electrophysiological and other mapping techniques have provided important insights into the function of neural circuits and neural populations in many systems. However, there remain limitations with these approaches. Therefore, complementary techniques which permit the monitoring of the spatio-temporal activity in neuronal populations are of continued interest. One promising approach to monitor the electrical activity in populations of neurons or on multiple sites of a single neuron is with optical recording techniques using voltage-sensitive dyes. One obvious advantage is the possibility of simultaneous measurements from many locations, while the system under study remains intact. Voltage sensitive dyes are fast organic potentiometric markers, which indicate changes in membrane potential, thus rendering them a widely used tool in neurophysiology and relative pathological conditions, such as epilepsy. Epilepsy is a severe neurological disorder characterized by recurrent convulsive or non convulsive episodes, reflecting massive neuronal discharges with rate of incidence up to 3% worldwide.
In this study, we made an in vitro epilepsy model based on acute coronal hippocampal slices (from mice) perfused in Mg2+-free or in 4-amynopiridine (100μM, 4-AP) containing artificial cerebrospinal fluid (ACSF) provoking spontaneous seizure-like events. Prior to perfusion, slices were stained with the voltage-sensitive dye di-8-ANEPPS. A high-speed CMOS camera connected to the c-mount port of an upright epifluorescence microscope was used for optical recordings. Aiming in elucidating the role of inhibitory (GABAergic) neuorotransmission in hippocampal excitability, we performed pharmacological interventions using the GABAA antagonist, biccuculine (60μM). It came out that it is possible to obtain optical recording of spontaneous epileptic activity, thus providing clear evidence of the approach’s feasibility.
Research reported in this poster was supported by the Hellenic Foundation for Research and Innovation (H.F.R.I.) under the “First Call for H.F.R.I. Research Projects to support Faculty members and Researchers and the procurement of high-cost research equipment grant” (Project Number: 286)."

54. Investigating the role of membrane potential dynamics in cancer cell proliferation using optogenetics

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Cancer cells generally exhibit a depolarized phenotype and are characterized by overexpression of ion channels on their plasma membrane. Ion channels and pumps are finely tuned to regulate the voltage gradient across the plasma membrane and therefore determine the membrane potential (Vm). Numerous studies have provided evidence for a regulatory role of the Vm changes in cellular processes such as proliferation. It has also been observed that activation of receptors by mitogenic stimuli leads to Vm changes. Electrical signals could be transmitted from the plasma membrane to other internal organelles and eventually to the nucleus where they may regulate classical biochemical signaling cascades. It is postulated that this transduction is mediated by the endoplasmic reticulum (ER). The ER contacts every other organelle in the cell including the nucleus and the plasma membrane, therefore it could act as a transmitter of electrical signals. However, the functional role of this mechanism remains unclear since the Vm of the ER and intracellular electrical phenomena cannot be directly measured by electrodes.
The focus of this study is to implement optogenetic tools and optical recordings into cancer cells, to investigate membrane potential dynamics of the plasma membrane and the ER. Optogenetics provide the means to record changes in cell-type specific populations with great spatiotemporal resolution. In particular, experiments on HEK 293T were performed using the voltage-sensitive dye di-8-ANEPPS to optically record voltage changes in response to field stimulation. Square pulses of 40mV and 100ms duration were used. Optical signals were recorded and the fluorescence change ΔF/F was measured. The cell population segregated into depolarizing and hyperpolarizing groups in a spatiotemporal manner. Pharmacological manipulations using Carbenoxolone and 4-Aminopyridine followed to investigate electrical signal propagation and explore role of gap junctions."

55. Modulation of Nrf2/Keap1 signalling pathway by hydroxytyrosol biosynthesized from genetically modified Escherichia Coli strains

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Background Hydroxytyrosol (HT), a natural polyphenolic compound of olive oil is a powerful antioxidant that scavenges free radicals, stimulates the synthesis and activity of endogenous antioxidant enzymes, and reduces lipid peroxidation. The extraction of HT from natural sources is an expensive process; thus, cheap, and reliable methods that can produce high amounts of pure HT with minimal cost are needed.
Aim The evaluation of the in vitro biological effects of HT biosynthesised from genetically modified E. Coli strains.
Methods The cytotoxic activity of HT against HeLa cells was assessed by means of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Flow cytometry was used for the estimation of apoptosis (Annexin-FITC and Propidium Iodide staining) and intracellular reactive oxygen species (ROS) (DCFDA staining). Western blotting of cell extracts was executed for the detection of the protein expression of the key molecules of the Nrf2/Keap1 signalling pathway.
Results HT exerted dose-dependent cytotoxicity against HeLa cells. The IC50 values were estimated at 41 μg/ml and 22 μg/ml after 24 and 48 hours, respectively. Moreover, HT induced apoptotic cell death, especially at concentrations higher than 10 μg/ml. Preincubation of HeLa cells with HT resulted in a dose-dependent decrease in ROS formation. Finally, HT affected cells by causing the activation of the Nrf2/HO-1 signalling pathway, possibly providing, thereby, cytoprotection against oxidative stress.
Conclusion HT produced from genetically modified Escherichia Coli strains boosted the antioxidant potential of HeLa cells. HT caused the Nrf2 and HO-1 proteins induction and the activation of the Keap1/Nrf2/Ho-1 pathway allowing cells to protect against severe levels of oxidative stress.

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56. In vivo antioxidant effects of hydroxytyrosol biosynthesized from genetically modified Escherichia Coli strains

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Background Hydroxytyrosol (HT) is a natural polyphenolic compound of olive oil with a wide biological role. The extraction of HT from natural sources (olive oil products) is an expensive process. Different bacteria strains, as well as substrates, are used to produce highly pure HT through the application of biotechnological approaches.

Aim The evaluation of the in vivo biological effects of HT biosynthesised from genetically modified E. Coli strains

Methods Thirty CD-1 mice, randomly divided into 3 groups were used. HT was diluted into mice drinking water and supplementation lasted 90 days. The activity of catalase as well glutathione levels in mice plasma were determined utilizing ELISA assay. For the detection of the protein expression of the key molecules of the Nrf2/Keap1 signalling pathway, analysis with Western blotting of hepatic tissue extracts was performed.

Results Catalase activity remained constant throughout the supplementation period. At the end of the study, a significant decrease ($p<0.05$) was noted in glutathione levels for both HT-supplemented groups. Furthermore, the positive effect of HT on the hepatic tissues of mice and the possible activation of the Nrf2/HO-1 signalling pathway with the potential release and translocation of Nrf2 to the nucleus was also noted.

Conclusion Chronic oral supplementation with HT strengthened the antioxidant potential of the hepatic tissue. Nonetheless, the mild increase in catalase activity and the decrease in glutathione levels in mice plasma warrants further investigation to identify a potential multifactorial effect of HT on the antioxidant network regulation.

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57. The effects of hydroxytyrosol on mice embryonic cells that overexpress the human heat shock protein 70

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Background The Mediterranean diet is known for its beneficial health effects, mainly due to the consumption of olive oil. Despite the high content of fatty acids, olive oil's beneficial result comes from antioxidant molecules, such as hydroxytyrosol (HT) which bind and deactivate reactive oxygen species (ROS). Heat shock protein 70 (Hsp70) is a molecular chaperone that has a crucial role in protein folding, degradation and translocation and participates in single-strand DNA repairing and thus has an influence on cell viability and apoptosis. Moreover, Hsp70 through the regulation of several intracellular proteins and signalling pathways forms a complicated antioxidant network that regulates redox homeostasis.

Aim To examine the cytotoxic and antioxidant activity of HT in NIH/3T3 cells and mice embryonic cells that overexpress the human Hsp70 (Tg/Tg cells)

Methods The cytotoxic activity of HT against NIH/3T3 and Tg/Tg cells was assessed employing 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and clonogenic assays and ROS were quantified with flow cytometry (2',7'-dichlorofluorescin-diacetate staining).

Results HT exerted a similar dose-dependent reduction of cell viability to NIH/3T3 and TgTg cells after 24 hours of treatment. However, after 48 hours the cytotoxic effect of HT was more profound to TgTg than NIH/3T3 cells at doses lower than 20 μg/ml. Pre-incubation of cells with HT boosted the antioxidant capability of NIH/3T3 cells who were able to reduce H2O2-induced ROS formation by more than 50%. On TgTg cells, HT failed to enhance their scavenging ability to ROS.

Conclusion TgTg cells were more sensitive to the cytotoxic activity of HT than NIH/3T3 cells. Moreover, HT antioxidant effect was only visible in NIH/3T3 cells possible indicating that TgTg cells are more resistant to ROS detrimental effects. So far, data on the ability of HT to modulate HSP70 are scarce. Further research in TgTg cells could shade more light on the effect of HT on"

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58. PRENATAL ALCOHOL EXPOSURE AND EXPERIMENTAL SEIZURE SEVERITY IN GROWING AND ADULT RATS

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Prenatal alcohol exposure (PAE) has been linked to developmental neurological disorders, including an increased risk of seizures, a correlation not widely studied, particularly in mild PAE models. We investigated the effects of mild PAE in seizure susceptibility & severity of the offspring, using a model that has shown neuronal excitability changes in vitro in our lab.
Methods: Young, adult nulliparous female Sprague-Dawley rats received an ethanol solution as their drinking water for 2 weeks (first 10% v/v, then 15% v/v in water) before breeding and throughout gestation. After parturition, the ethanol concentration was gradually decreased and eventually replaced by clean water on the 15th day. Control (Normal, N) offsprings were obtained from dams who had not been exposed to alcohol. To induce seizures, GABAA antagonist Pentylenetetrazol was systemically administered via i.p. injections at either postnatal day 20 (Young) or postnatal day 60 (Adult) on both Normal and PAE rats, until generalized tonic-clonic seizures were observed. A new classification scale was devised based on previously published ones* to accurately define seizure behavior (""stages,"" ""characteristics""). Fisher's exact test (or Chi-square) and student's t-test (unpaired samples, Prism program) were used to statistically analyze the data.
Preliminary data analysis showed that this model of PAE increased mildly experimental seizure severity in Y but not in A animals, suggesting considerable powers of developmental adaptation. Specifically, more PAE animals reached stages 7 and 8 compared to N of the same age and gender and also stage 6 duration was significantly longer in Y PAE animals compared to N. Nevertheless, increased seizure severity in Y PAE animals may presumably lead to (permanent ?) long term effects in aspects of CNS function.
*(Racine 1972, Pinel & Rovner 1978, Lüttjohann et al. 2009, Velíšková & Velíšek 2017, Jan Van Erum et al. 2019)"

59. Natural Killer T cells (NKT) contribute to Hepatocellular Carcinoma

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Hepatocellular Carcinoma (HCC) is a leading cause of death in the West with high mortality due to late-stage diagnosis. Liver is prone to HCC development due to its immunosuppressive environment. In HCC immunology, immune cells that fight the tumor such as Natural Killers (NKs), cytotoxic T cells (CD8 T cells), dendritic cells (DCs) are suppressed, while the function of immune cells such as regulatory T cells (Tregs), tumor associated macrophages (TAMs) that promote tumor growth is enhanced. Natural Killer T cells (NKTs) are tissue resident cells that are largely enriched in the liver. NKT cells are a subpopulation of T cells that have characteristics of both innate and adaptive immunity. They recognize lipid, not peptide, antigens through the CD1d molecule, and exit the thymus in a pre-activated state, unlike naïve conventional T cells. As a result, they can produce large amounts of various cytokines and chemokines that control the immune response. However, the role of NKT cells in HCC is not clear. To dissect the functions of NKT cells, we chemically induced HCC after DEN treatment in WT, CD1d-/- and Vα14Tg/+ mice. Our preliminary data show that HCC development was delayed in mice that do not produce NKT cells, while it was not affected in mice that overproduce NKT cells. NKT cells were significantly reduced in WT and Va14Tg/+ mice with HCC compared to controls and their function was impaired. After short-term DEN treatment, CD1d-/- mice showed reduced liver damage than WT mice, according to ALT and AST levels, and inflammation was resolved faster. Therefore, we suggest that NKT cells contribute to the initiation and/or progression of HCC.

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60. IMMUNE MEDIATED RESPONSES AGAINST NEURAL PRECURSOR CELLS AFTER TRANSPLANTATION IN EXPERIMENTAL ANIMAL MODEL OF MULTIPLE SCLEROSIS

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PURPOSE OF STUDY:

Multiple sclerosis (MS) is a chronic autoimmune demyelinating disorder of the central nervous system (CNS) with undetermined pathophysiology. Recent studies suggest the transplantation of neural precursor cells (NPCs) in experimental autoimmune encephalomyelitis (EAE), an experimental animal model of MS, as a potential therapeutic approach. The purpose of the present study is to investigate immune mediated responses triggered by transplantation of NPCs.

MATERIALS AND METHODS:

The research project was conducted in 6-8 weeks old female C57BL/6 mice and consisted of five experimental groups: A) control group (n=7), B) group that has been inoculated only with complete Freund's adjuvant (CFA) (n=7), C) EAE group (n=7), D) EAE with intrathecal injection of NPCs (n=7) and E) naive mice with subcutaneous paralumbar inoculation of NPCs (n=7). Blood sampling was performed on day 50 (chronic phase) and corresponding antisera were collected (NAIVE-AS, CFA-AS, EAE-AS, ITH-AS, NPCs-AS) and examined for the presence of autoantibodies by western blotting (WB) in NPCs substrate.

RESULTS: WB revealed that EAE mice and mice immunized with NPCs exhibited repetitive patterns in the recognition of specific protein bands in the antisera. Specific patterns with NPCs substrate were also found in the antisera of the EAE mice that were intrathecally transplanted. Common repetitive patterns were identified among the antisera from transplanted and immunized mice (~100kDa, ~75kDa, ~60kDa, ~48kDa, ~30kDa).

CONCLUSION:

In conclusion, the present study suggests that NPCs, a promising cell therapy for neurodegenerative diseases including MS, which were previously considered to be immune privileged, may actually trigger immune mediated responses after their administration. Therefore, the immunogenic potential of NPCs makes imperative the need to further delineate the exact mechanisms of these immune responses in order to evaluate their safety and efficiency of administration."

61. The syndrome of frailty: The modern “Achilles heel”

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Introduction
Our objective is to review the current literature concerning the syndrome of frailty, its complications and potential therapeutic approach.
Methods
We conducted a PubMed search to identify all relevant publications regarding our topic.
Results
Frailty is the pedestal of geriatric medicine. The progressive aging of the population made its presence visible. It is characterized as a clinical condition related to excessive vulnerability to both endogenous and exogenous stressors, along with decreased homeostatic reserves. The cycle of frailty, as described by Fried and Walston, consists of five components: weakness, slowness, exhaustion, weight loss and low activity (frailty phenotype), whilst sarcopenia, which is related with muscle mass loss is one of the basic components of this syndrome, apart from malnutrition, inactivity, social isolation and polypharmacy. A number of frailty indexes has been proposed to assess frailty, such as Clinical Frailty Scale (CFS) and FRAIL scale. Its complications concern falls and fractures, disabilities, hospitalizations, increased healthcare cost and premature mortality. Interventions in order to prevent its vice outcomes may include exercise programs and appropriate nutrient intakes.
Conclusion
The review of the current literature highlights the diagnostic significance of frailty syndrome in order to eliminate or reduce its complications and to provide a better quality of life for individuals.
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62. THE PHYSIOLOGICAL ADAPTATIONS IN BASKETBALL ATHLETES FROM THE PREPARATION TO THE SEASON'S END

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ABSTRACT

Basketball requires a high response from both the anaerobic and aerobic energy production mechanisms. Therefore, this study aims to investigate the physiological adaptations of athletes during an entire season. Measurements were recorded on fifty-four Greek League's 2 basketball players in four different periods (before and after the preparation, in the middle and at the end of the championship). The evaluation included anthropometric characteristics, cardiorespiratory parameters and leg explosive and elastic power. The researchers used an ergo-spirometer connected to a treadmill and a wireless transmitter, calibrating the instruments every five measurements. In addition, a force platform equipped with piezoelectric sensors was used for the leg power. The results showed statistically significant changes in weight, body fat percentage, maximal oxygen uptake capacity (VO2max), the velocity at maximal oxygen uptake and anaerobic threshold up to the middle of the championship. At the same time, there was a decrease in the variation of the maximum heart rate. Finally, the explosive and elastic force jump ability was increased. However, there was a decrease in performance during the last measurement at the end of the season (40th week). In conclusion, recording, analyzing and studying the physiological adaptations can contribute to designing more effective and targeted training for the individual athlete's needs.

KEYWORDS: BASKETBALL, CARDIORESPIRATORY PARAMETERS, LEG POWER, PHYSIOLOGICAL CHARACTERISTICS."

63. Angiopoietin-2-induced lymphatic endothelial cell migration drives lymphangiogenesis via the β1 integrin-RhoA-formin axis

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Lymphangiogenesis is an essential physiological process but also a determining factor in vascular-related pathological conditions. Angiopoietin 2 (Ang2) plays an important role in lymphatic vascular development and function and its upregulation has been reported in several vascular-related diseases, including cancer. Given the established role of the small GTPase RhoA on cytoskeleton-dependent endothelial functions, we investigated the relationship between RhoA and Ang2-induced cellular activities. This study shows that Ang2-driven human dermal lymphatic endothelial cell (HDLEC) migration depends on RhoA. We demonstrate that Ang2-induced migration is independent of the Tie receptors, but dependent on β1 integrin-mediated RhoA activation with knockdown, pharmacological approaches, and protein sequencing experiments. Although the key proteins downstream of RhoA, Rho kinase (ROCK) and myosin light chain (MLC), were activated, blockade of ROCK did not abrogate the Ang2-driven migratory effect. However, formins, an alternative target of RhoA, were identified as key players, and especially FHOD1. The Ang2-RhoA relationship was explored in vivo, where lymphatic endothelial RhoA deficiency blocked Ang2-induced lymphangiogenesis, highlighting RhoA as an important target for anti-lymphangiogenic treatments.