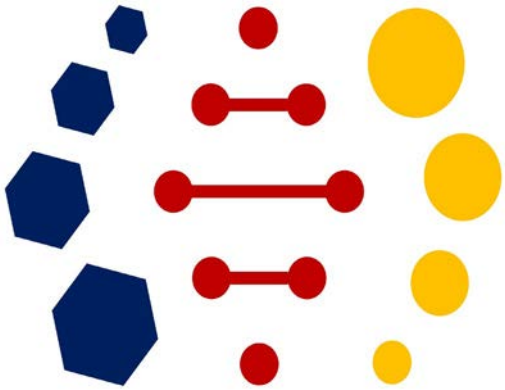

IV Metabolism, Ageing & Disease Meeting

Disease & Therapeutics: from the Past to Future

Program

Coimbra, Portugal | 29-30 January, 2024

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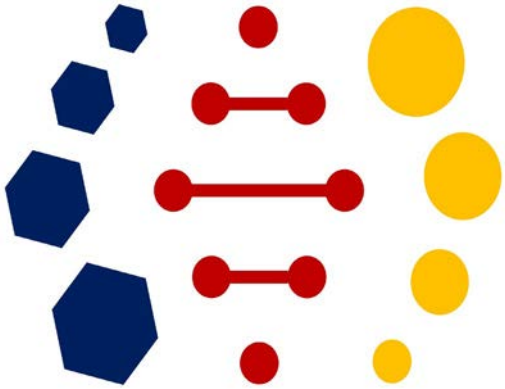
18h00	**Awards & Closing**



IV Metabolism, Ageing & Disease Meeting

Disease & Therapeutics: from the Past to Future

Abstract Book

Coimbra, Portugal | 29-30 January, 2024

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Welcome to IV MAD meeting

Disease & Therapeutics: from the Past to Future

We're excited to receive you in the **MAD Meeting 2024,** taking place between 29th and 30th January 2024 in the Auditorium at the subunit 3, Faculty of Medicine (Polo III), University of Coimbra. During these two days we will have keynote talks, invited talks, flash talks and posters.

We will have 3 prizes for the first- and second-best flash talks, and the best poster, that will be announced during the closing ceremony.

All this was only possible due to the Institutional support and the sponsors. So, we would like to thank the Institutional Support given by CIBB, CNC, iCBR, the Faculty of Medicine of The University of Coimbra and the Institute for Interdisciplinary Research of the University of Coimbra.

We would like to give a special thanks to the generosity of our Sponsors! In alphabetical order, thank you very much to: Alfagene, Centogene, Enzifarma, LaborSpirit, Novartis, Pfizer, PTC Therapeutics, Roche, Sandoz, Soquímica, Specanalítica, The Company of Biologists.

Now, we invite you to share your work and to network with fellow researchers. We look forward to offering you an exciting time, where the past and future of Disease & Therapeutics will come together in one exciting event.

The Organizing Committee

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Organizing Commission

Ana Cristina Gonçalves
Célia Cabral
Ermelindo Leal
Filipe Palavra
José Teixeira
Judy Paulo
Raquel Alves
Susana Pereira

Scientific Commission

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Metabolism, Ageing and Disease · Centre for Innovative Biomedicine and Biotechnology

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ICBR Coimbra Institute for Clinical and Biomedical Research

CEISUC CENTRO DE ESTUDOS E INVESTIGAÇÃO EM SAÚDE UNIVERSIDADE DE COIMBRA

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IV Metabolism, Ageing & Disease (MAD) Meeting

Keynote Talks

Interaction between liver metabolism and necroptosis

Cecília M. P. Rodrigues [1,*]

1 Research Institute for Medicines (iMed.ULisboa), Fcaulty of Pharmacy, Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisbon, Portugal

*Correspondence: cmprodrigues@ff.ulisboa.pt

Abstract:

Non-alcoholic fatty liver disease (NAFLD), recently renamed metabolic dysfunction-associated steatotic liver disease (MASLD) affects more than 25% of the adult population paralleling obesity and diabetes. Despite the lack of approved therapies, pharmacological inhibition of receptor-interacting protein kinases (RIPK) improves experimental MASLD in part by increasing mitochondrial respiration (Majdi *et al. J Hepatol.* 2020). In addition, mitofusin 2 is decreased in liver biopsies from MASLD patients and its deficiency reduces phosphatidylserine transfer and phospholipid synthesis, leading to ER stress and inflammation (Hernandez-Alvarez *et al. Cell.* 2019). RIPK3 is a key player in necroptosis and an emerging metabolic regulator, whose contribution to MASLD is controversial. We have shown that hepatic RIPK3 increases in patients with MASLD, correlating with hepatic inflammation and fibrosis (Afonso *et al. Gut.* 2021). Accordingly, *Ripk3* deficiency ameliorated diet-induced hepatocellular damage, inflammation and fibrosis in a mouse model that recapitulates human MASLD. Furthermore, *Ripk*3 deficiency hampered hepatocarcinogenesis. Intriguingly, deletion of *Ripk3* increased liver fat accumulation and shifted the hepatic lipidome *in vivo*, although lipid droplets were smaller in fat-loaded *Ripk3*$^{-/-}$ hepatocytes (Afonso *et al. Hepatology* 2023). *Ripk3* deficiency upregulated PLIN1 and 5, which in turn are implicated in mitochondria-lipid droplet interactions. In line, *Ripk3* deficiency rescued mitochondrial biogenesis, bioenergetics and function *in vivo* and *in vitro.* Data from preclinical models and patients indicate that the PGC-1α/PPARγ/PLIN1 axis is functionally implicated in improving lipid and mitochondrial metabolic homeostasis by *Ripk3* deficiency. Conversely, a pathogenic *PLIN1* frameshift variant was associated with MASLD, fibrosis, and RIPK3 levels in familial partial lipodystrophy. In conclusion, RIPK3 plays a key role in managing liver metabolism, damage and carcinogenesis, whereby RIPK3 inhibition may ameliorate MASLD.

Acknowledgment: Funding from H2020 IMI2 Litmus, MSCA ITN Foie Gras; La Caixa Foundation; FCT

Neuroimmune Regulation of Metabolism

Henrique Veiga-Fernandes [1,*]

1 Champalimaud Research, Champalimaud Centre for the Unknown, Av. Brasília, 1400-038 Lisboa, Portugal

*Correspondence: henrique.veigafernandes@research.fchampalimaud.org

Abstract:

Neuroimmune interactions have been revealed to be at the centre-stage of tissue defence and organ homeostasis. Neuronal and immune cell subsets can colocalise in discrete tissue environments, forming neuroimmune cell units that constitute the basis for bidirectional interactions and which drive coordinated neuroimmune responses to local and systemic challenges. Nevertheless, whether neuronal and immune cells cooperate in inter-organ communication axes to orchestrate organismal health and metabolic homeostasis remains elusive. Here, we will discuss how neuroimmune circuits integrate and respond to their environment to regulate metabolic homeostasis, in health and disease.

I&D projects in the field of innovative processes for natural ingredients production

Lillian Barros

1 Centro de Investigação de Montanha (CIMO), Instituto Politécnico de Bragança, Campus de Santa Apolónia, Bragança, Portugal; 2 Laboratório Associado para a Sustentabilidade e Tecnologia em Regiões de Montanha (SusTEC), Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal

* Correspondence: lillian@ipb.pt

Abstract:

Recently, scientific validation of an array of applications and benefits arising from the use of natural ingredients and edible matrices has increased worldwide. Here, the improvement of health conditions through the use of plants and mushrooms represents a rich cultural legacy, with these matrices being traditionally used as sources of micro and macronutrients and in different medicinal preparations. These are made up of natural ingredients with high added value, acting as natural colorants and preservatives, and providing bioactive properties when added to other products.

Currently, various innovative technologies have been used to optimize extraction systems, increasing the purity of the natural target compounds and extraction yields. Some colorants have been successfully extracted from various natural sources, and used in different food formulations. On the other hand, a wide variety of biowastes and/or by-products have been effectively used as bioactive molecules, such as those from mushrooms and fruit residues, which have shown to perform different bioactivities. Also biowaste from the primary and transformation production have been used as preservatives and also used in the formulation of healthier food formulations. Additionally, in this field, the use of figs and pumpkins through the extraction of bioactive compounds from all their components and subsequent incorporation into derived products, show satisfactory outcomes, boosting the circular economy of all its constituents. In parallel, bioactive molecules from other biowastes have been recovered for further incorporation into cosmeceutical formulations, while improving sustainable extraction processes and their optimization.

These results have been accomplished due to a diversity of different financed research projects, which allowed to highpoint the effectiveness of natural ingredients from different natural matrices, promoting their valorisation as sources of naturally based ingredients able to be incorporated into widely consumed and appreciated food products at an industrial/commercial level.

Acknowledgment: The authors are grateful to the Foundation for Science and Technology (FCT, Portugal) for financial support through national funds FCT/MCTES (PIDDAC) to CIMO (UIDB/00690/2020 and UIDP/00690/2020) and SusTEC (LA/P/0007/2020). National funding by FCT, through the scientific employment program-contract for L. Barros (CEEC Inst.) contract.

K04

Unveiling the Significance of Non-Coding RNAs in Liquid Biopsy: A Quest for Urological Cancer Biomarker Discovery

Carmen Jerónimo [1,2,3]*

1 Cancer Biology & Epigenetics Group, Research Center of IPO Porto (CI-IPOP)/RISE@CI-IPOP (Health Research Network), Portuguese Oncology Institute of Porto (IPO Porto)/Porto Comprehensive Cancer Center Raquel Seruca (Porto.CCC), R. Dr. António Bernardino de Almeida, 4200-072 Porto, Portugal; 2 Department of Pathology, Portuguese Oncology Institute of Porto (IPO Porto)/Porto Comprehensive Cancer Center Raquel Seruca (Porto.CCC), R. Dr. António Bernardino de Almeida, 4200-072 Porto, Portugal; 3 Department of Pathology and Molecular Immunology, ICBAS–School of Medicine and Biomedical Sciences, University of Porto, Rua Jorge Viterbo Ferreira 228, 4050-513 Porto, Portugal

*Correspondence: cljeronimo@icbas.up.pt

Abstract:

In the quest against cancer, elevating patients' survival rates is a societal imperative. This commitment is evident in many endeavours dedicated to crafting effective biomarkers for timely cancer identification. Early cancer detection significantly enhances the likelihood of successful treatment, sidestepping the need for subsequent therapies with their attendant side effects and health complications. Moreover, the existing criteria for categorizing cancer patients have revealed diverse outcomes. Hence, the pursuit of novel biomarkers, preferably discernible through minimally invasive techniques, holds pivotal significance, promising a profound impact on cancer detection and prognosis.

Liquid biopsies have emerged as promising tools, capturing considerable attention over the last decade. MicroRNAs have shown notable potential in advancing cancer diagnosis and patient management within the spectrum of biomarkers derived from biofluids.

During my presentation, I will delve into the recent significant findings from our research team on the role of microRNAs as biomarkers for detecting and prognosticating urological cancers. Additionally, I will address the current challenges associated with their implementation in clinical settings.

Stress Neuromatrix

Nuno Sousa [1,2,*]

1 Life and Health Sciences Research Institute (ICVS), School of Medicine, Campus Gualtar, University of Minho, 4710-057 Braga, Portugal; 2 ICVS/3B's-PT Government Associate Laboratory, 4806-909 Guimarães, Portugal

*Correspondence: njcsousa@med.uminho.pt

Abstract:

Stress is part of life. Stressful stimuli activate a consistent and reproducible set of brain regions; yet, the notion that there is a single and constant stress neuromatrix is not sustainable. Indeed, after chronic stress exposure there is activation of many brain regions outside that network. This suggests that there is a distinction between the acute and the chronic stress neuromatrix. In this talk, a new working model is proposed to understand the shift between these networks. The understanding of the factors that modulate these networks and their interplay will allow for a more comprehensive and holistic perspective of how the brain shifts 'back and forth' from a healthy to a stressed pattern and, ultimately, how the latter can be a trigger for several neurological and psychiatric conditions.

Ageing@Coimbra and CHAngeing Excellence Hubs: connecting dots to support science excellence, innovation and societal impact in health and longevity in the Center Region of Portugal

João Malva* [1]

1 Coimbra Institute for Clinical and Biomedical Research (iCBR), Institute of Pharmacology and Experimental Therapeutics, Faculty of Medicine and Center for Innovative Biomedicine and Biotechnology (CIBB), University of Coimbra, 3000-548 Coimbra, Portugal,
*Correspondence: jomalva@fmed.uc.pt

Abstract:

Portugal faces a major socioeconomic challenge related with demographic changes and population ageing. In fact, Portugal, and specifically the Center Region, is among the regions facing most fast double ageing involving increased number of older adults and decreased number of citizens at young ages. This societal challenge is boosted by the poor quality of life of older adults, frequently suffering from multimorbid chronic conditions and physical/cognitive frailty limiting functional independence and dignity for several years at retirement ages. Portuguese are among the European citizens with longer life expectancy but, unfortunately, with poor quality of life and lower number of expected healthy years after 65.

These sociodemographic figures are key to highlight the need for innovative responses to the needs created by population ageing. Innovation and excellence science are key in this process and stand as pillars for science translation and development of good practices to support healthy living and longevity.

Ageing@Coimbra was founded in 2013 as a need to create a regional holistic collaborative network based on the concept of the quadruple helix to develop, replicate and scale-up innovative good practices to support individuals, families and the society in building responses for the present and future challenges related with population ageing. This movement has been inspired by excellence science and by the need to create conditions to leverage the impact of excellence science at regional, national and European/Global scale. This vision created recognition by Regional and National Authorities and by the European Commission and was the basis to attract flagship projects like EIT Health, Teaming MIA-Portugal, Era Chair, ERA@UC, Twinning ReseatAging, EIT Health Ageing PhD School and recently Horizon Europe Excellence Hubs CHAngeing, among others. CHAngeing is a collaborative network between Ageing@Coimbra partners (Center Region of Portugal) and FORTH (Crete, Greece) base on the benefits of Mediterranean Diet Culture to support healthy lifestyles.

Acknowledgment: CHANgeing Excellenece Hubs is supported by the European Union, reference HORIZON-WIDERA-2022-ACCESS-04-01, project 101087071

IV Metabolism, Ageing & Disease (MAD) Meeting

Invited Talks

The power within: AntiOxCIN4 reduces oxidative/nitrosative stress in transgenic *SOD1*^{G93A} mice for amyotrophic lateral sclerosis

Débora Mena[1,2,3], Fernando Cagide[4], Sofia Benfeito[4], Katarzyna Michalik[1,5], Luís Grilo[1,2,3], Daniela F. Silva[1], Paulo Pinheiro[1], Elisabete Ferreiro[1,2], José Teixeira[1,2], Filomena Silva[1], Fernanda Borges[4], Paulo J. Oliveira[1,2,6] and Ana I. Duarte[1,2,6,7,8*]

1 CNC-UC – Ctr Neurosci Cell Biol, Univ Coimbra, Coimbra, Portugal. 2 CIBB - Ctr Innov Biomed Biotechnol, Univ Coimbra, Portugal. 3 PDBEB - PhD Program Exp Biol Biomed, Inst Interdiscipl Res (IIIUC), Univ Coimbra, Portugal. 4 CIQUP-IMS/Dept Chem Biochem, Fac Sci, Univ Porto, Porto, Portugal. 5 Dpt Mol Physiol Neurobiol. Univ Wroclaw, Wroclaw, Poland. 6 IIIUC, Univ Coimbra, Portugal. 7 Inst Pharmacol Exp Therap, Fac Med, Univ Coimbra, Portugal. 8 iCBR - Coimbra Inst Clin Biomed Res, Fac Med, Univ Coimbra, Portugal.

* Correspondence: amduarte@fmed.uc.pt

Abstract:

Amyotrophic lateral sclerosis (ALS) is a fatal motor and neurodegenerative disease. Mitochondrial dysfunction and oxidative stress may play a pivotal role in its pathophysiology. Thus, rescuing mitochondrial function and/or antioxidant defenses with novel mitochondriotropic antioxidants, like AntiOxCIN4, may be beneficial against ALS. We hypothesized that AntiOxCIN4 can protect against oxidative/nitrosative stress in the brain and skeletal muscle of *SOD1*^{G93A} ALS mice.

SOD1^{G93A} ALS mice were subcutaneously injected with AntiOxCIN4 (0.1 mg/Kg/day), for 2 months. We evaluated mouse longevity, and brain and skeletal muscle oxidative/nitrosative stress, superoxide dismutase (total SOD and SOD-2) and glutathione reductase (GRed)) activities.

AntiOxCIN4 slightly recovered female ALS mice longevity and neuromuscular function. It slightly decreased their skeletal muscle total SOD and SOD-2 activities, and upregulated GRed activity. AntiOxCIN4 also decreased ALS skeletal muscle hydroperoxides and nitrites levels. AntiOxCIN4 (slightly) upregulated the activities of antioxidant enzymes and decreased nitrites levels in ALS brains.

In sum, AntiOxCIN4-related increased longevity in ALS female mice may arise from the protection against skeletal muscle and brain oxidative/nitrosative stress. However, further clarification is needed.

Acknowledgment: Funded by European Regional Development Fund: *Centro2020 Operational Programme* (POCI-01-0145-FEDER-029391 (*Mito4ALS*)), *COMPETE 2020*; by Portuguese Fundação para a Ciência e a Tecnologia (FCT): POCI-01-0145-FEDER-029391, PTDC/MED-FAR/29391/2017, UIDB/04539/2020, UIDP/04539/2020, LA/P/0058/2020, UIDB/00081/2020; by European Social Fund: 2021.04707.BD & Mito4ALS-PTDC/MED-FAR/29391/2017 (DM), DL57/2016 (FC); FCT/P2020/COMPETE, PTDC/MED-QUI/29164/2017 (SB); SFRH/BD/5539/2020 (LFG); ERASMUS+ Student Mobility Grant (KM); 2020.01560.CEECIND (JT); DOI: 10.54499/DL57/2016/CP1448/CT0006 & EU HORIZON-WIDERA-2022-ACCESS-04-01 Excellence Hubs/CHAngeing/101087071/II0347.01 (AID).

Decoding the diabetic brain: Unveiling the Known and Unknown

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Abstract:

Type 2 diabetes (T2D) has attained the status of a global pandemic becoming one of the major health challenges of the 21st century. Long gone is the idea of a western disease with the far-ranging and rapid socio-economic changes worldwide playing a key role in "spreading" the disease in the past few decades. Unhealthy lifestyle habits, including high calorie diets (HCD) and lack of physical activity, are responsible for an increase in metabolic disorders namely T2D and/or obesity. With the increase in T2D burden also its well-known associated complications have increased posing a considerable burden on millions of people, with devastating health consequences and a massive impact on the global economy. Among them, T2D-associated neurodegeneration comprises one of the leading causes of disability and death in people with T2D and, strong evidence demonstrates a link between T2D and cognitive decline. However, serious knowledge gaps on how T2D affects the brain remain.

In the last years, our group has been focused in unveil the mysteries behind the diabetic brain, specifically how T2D affects brain mitochondria and their potential as mechanistic targets to counteract diabetes-associated neurodegeneration. Using mouse models of the disease, we have clearly shown that T2D and AD share common features such as mitochondria defects, oxidative stress, vascular defects and inflammation, which contributed to a similar profile of behavioural and cognitive anomalies. However, about 99.6% of clinical trials, including those targeting energy metabolism, have failed to exert disease-modifying efficacy probably due to a "to late" intervention. In this line, it has been recently shown that WW domain-containing oxidoreductase 1 (WWOX) overexpression/overactivation plays a pivotal role in mitochondrial dysfunction and development of insulin resistance, features shared by T2D and AD. Thus, we hypothesized that WWOX may represent a key therapeutic target to take in account, acting upstream mitochondria. Our recent studies demonstrate that WWOX protein triggers mitochondrial dysfunction contributing to hyperglycemia-induced neuronal damage and diabetic brain defects. More, we clearly showed the therapeutic potential of Zfra1-31, its specific inhibitor, against diabetes-associated brain damage.

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Endometrial cancer stem cells: a translational research

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Abstract:

Endometrial cancer (EC) is the most frequent gynecological malignant disease in developed countries[1]. For early-stage EC, the first-line treatment is surgery, which in young women compromises their fertility. Besides frequent recurrence, a limitation of current conservative therapies is the need for surgical staging, which is based on sentinel lymph node (SLN) microstaging. A potential conservative approach could be photodynamic therapy, which shows selectivity to tumoral tissue, and their action, mediated by light irradiation of precise sites, avoids common adverse effects. Aiming for improved selectivity and staging alternatives, cancer stem cells (CSC) and their markers can be of paramount relevance. In our research, we identified endometrial-CSC, showing their self-renewal, differentiation potential, tumorigenesis, and oxidative metabolism. Among markers, aldehyde dehydrogenase (ALDH) stood out as having a preponderant role in EC CSC[2]. Still, to identify further key molecules, we are investigating the endometrial-CSC proteomic profile. A group of 5-tetrahydropyrazolo[1,5-α] pyridine fused chlorins[2-4] were modulated to be ALDH substracts (named A-Px) in an elegant strategy that boots photosensitizer accumulation in ALDH overexpressing cells. We saw that A-Px can internalize in endometrial CSC-enriched populations and exert a photodynamic effect. As a predictive stratification strategy, CSC were investigated in metastatic SLN. Preliminary studies from 12 EC patients showed a reduction in the hormone receptors expression in metastatic disease, which suggests dedifferentiation associated with a stemness profile. ALDH1 expression in macrometastasis was with CSC. Soon, we intend to investigate clinical and pathological factors, stem cell markers *vs.* response to PDT and SLN metastasis. With this project, we intend for a minimally invasive treatment, in association with a PDT-based theragnostic staging strategy, able to contribute to the decrease of morbidity, recurrence, and mortality rates and promote health quality and well-being.

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Dopamine, adipose tissue metabolism and obesity

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Abstract:

Dopamine is a key regulator of glucose metabolism in the central nervous system. The therapeutic effects of the dopamine D2 receptor (D2R) agonist, bromocriptine, in type 2 diabetes (T2D) have been attributed to central nervous system actions. However, peripheral dopamine directly modulates glucose uptake in insulin-sensitive tissues and lipid metabolism in adipose tissue (AT). Peripheral dopamine directly stimulates glucose uptake with its receptors being differentially involved in glucose uptake in insulin-sensitive tissues. Dopamine also has a role in lipid metabolism in white adipose tissue.

We demonstrated that the expression of dopamine receptors is reduced in the visceral adipose tissue of IR patients and correlated with InsR and metabolic mediators. In HFD-fed diabetic rats, Bromocriptine treatment remodels adipose tissue and the liver dopaminergic system, with increased D1R and TH levels, resulting in higher insulin sensitivity and catabolic function. Such effects on other dopaminergic machinery than D2R may also be involved in bromocriptine therapeutic effect.

Given that dopamine is secreted by the gut and regulates insulin secretion in the pancreas, we also aimed to determine its regulation by nutritional cues and its role in regulating glucagon-like peptide 1 (GLP-1) action in WAT. Postprandial dopamine levels showed elevations following a mixed meal and glucose intake. Bromocriptine treatment in the same model increased GLP-1R in WAT, showing the role of dopamine in regulating GLP-1R. Our results point out a dietary and gut regulation of plasma dopamine, acting in the WAT to regulate GLP-1 action.

Altogether, these results suggest that peripheral modulation of the dopaminergic system should be further evaluated as a putative therapeutic approach for metabolic disorders.

Unraveling the pre-metastatic niche: mechanistic insights for therapeutic interventions

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Abstract:

Metastasis is the leading cause of cancer morbidity and mortality, accounting for 90% of all cancer-related deaths. The development of distant metastasis is a highly complex process involving a sequence of dynamic events known as the metastatic cascade.

It has been shown that primary tumors prepare in advance and remotely a supportive and receptive microenvironment in specific secondary organs, referred to as premetastatic niches (PMNs), for incoming tumor cells to adapt and survive. Therapeutic strategies targeting PMN formation at organ-specific sites offer an opportunity to prevent or suppress metastasis formation and are currently a hot topic in cancer research. Identification of potential druggable targets requires a comprehensive tumor-specific understanding of the cellular and molecular mechanisms involved in establishing organ-specific PMNs.

We have been interested in understanding how osteosarcoma, a primary malignant bone tumor, reprograms the lung microenvironment to establish a permissive pro-metastatic niche for subsequent lung metastasis formation. Using murine models and a multi-omics approach, we uncovered neutrophil infiltration and the functional contribution of stromal-activated fibroblasts in extracellular matrix remodeling as early pro-metastatic events guiding lung metastasis. Furthermore, we identified EFEMP1, a glycoprotein secreted by tumor cells, as a potential driver of lung metastasis and a plasma biomarker with added value in predicting the risk of lung metastasis in osteosarcoma patients.

In addition, we found that osteosarcoma-derived small extracellular vesicles have a high affinity for homologous lung metastatic lesions, holding great potential as targeted imaging agents for the noninvasive detection of small lung metastasis by Positron Emission Tomography.

Turning waste into w(h)ealth: Next-gen prebiotics for immunometabolic diseases

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Abstract:

Polyphenols are a heterogenous chemical collection of plant secondary metabolites whose health-benefits are firmly demonstrated in a plethora of immunometabolic diseases. The biological properties of dietary polyphenols greatly rely on the bidirectional relationship with the gut microbiota (GM): while some classes of gut microorganisms are able to metabolize phenolic compounds, polyphenols can modulate GM composition and function while maintaining the intestinal barrier in normal status. Accordingly, they meet the criteria to be categorized as prebiotics in line with the last consensus on prebiotics' definition and scope. More recently, interest has shift toward understanding how gut microorganisms modulate the immune system, with a special emphasis on their interactions with the gut-associated lymphoid tissue (GALT) and the corresponding impact on host health, from oral tolerance maintenance to the biosynthesis of bacterial metabolites and toxins. Given the crucial role of polyphenols-GM reciprocal actions in maintaining host immunity, an emergent niche area of research relies on the development of next-generation prebiotics that may cumulatively fall in the scope of immunonutrition, a branch of precision nutrition seeking to selectively support immune responses, preventing and/or treating the risk for severity of an array of immune-mediated diseases.

The growing demand of polyphenols has led to the searching for new green sources being agrowaste an unconventional alternative for polyphenols recovery. Our ongoing work focuses on berry (BB) agrowaste upcycling towards the generation of next-generation prebiotics with immunonutritional value. We have developed an eco-friendly biotechnological approach for senescent BB leaves/stems processing and established a functionalized antioxidant fiber (BB biomass) with marked antioxidant, anti-dyslipidemic and prebiotic activities featured by decreased urinary nutrient-derived uremic toxins (e.g. indoxylsulfate) alongside increased levels of faecal SCFAs (C3-C5). In a safe oral dose (500mg/Kg/day, C56BL/6 mice), it reinforced intestinal mucus layer density and positively impacted GALT with respect to CD4+ T lymphocytes balance. Collectively, these results consubstantiate BB biomass prebiotic & immunonutritional effects. Now, we plan to further disclose BB biomass nutraceutical potential in immunometabolic diseases, both autoimmune (multiple sclerosis) and non-autoimmune (chronic kidney disease) mediated.

Male infertility: and when we don't know why?

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Abstract:

Currently affecting about 1 in 6 people worldwide, infertility is a disease with an alarming increasing trend that, for many couples, is more than the non-realization of essential human rights, namely, to decide if, when and how many children they want to father. In fact, this disease has a great social impact, not to mention the psychological distress withstood, which can further contribute to the couple's inability to conceive, and the heavy financial burden that it might imply. Male factor alone can contribute to up 20–30% of the cases of infertility, that when associated with female factors, rises to 50%. The main known causes for male infertility are urogenital and genetic anomalies, genital tract infections, endocrine disorders and immunological factors. However, even when the cause has been identified, the mechanisms behind it, and thus the identification of possible targets of intervention, are not well understood.

Adding to this scenario, some patients are diagnosed with unknown origin male infertility (UOMI). This categorization can be further divided into idiopathic (ID), and unexplained male infertility (UMI), which essentially differ in the seminal analysis results, abnormal in the former and normal in the later, assuming that in both the female factor has been ruled out. ID affects approximately 30 to 40% of infertile men, while UMI affects 6 to 30% of infertile men. In the literature, it is frequent to find different terms to classify these patients, which, together with the frequent lack of proper control groups and the fact that female factor is often disregarded, severely compromise the interpretation of the available information. Moreover, considering that the routine seminal analysis is the pillar for male infertility diagnosis, although its limitations in terms of predicting fertility are well-known, additional, or improved evaluation tools are needed to understand sperm functionality in full as also is a standard and more systematic assessment of potential risk factors, especially for UOMI patients. This stresses the need to deepen the study of these patients and their male gametes, ideally focusing on more relevant functional aspects, not routinely evaluated, but that might add knowledge on the mechanisms behind male infertility, hopefully providing the scaffold for the development of new diagnostic tools and treatment options. In my presentation, I will give an overview of my studies in this regard.

Connexins, exocytosis and extracellular vesicles

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Abstract:

Connexins are tetra-spanning membrane proteins that oligomerize to form channels at the plasma membrane. These channels directly connect the cytoplasm of cells with the extracellular milieu, or with the cytoplasm of adjacent cells. Although connexins were first recognized for their role in direct intercellular communication, we now know that connexins also possess other functions in the cell, including mitochondrial homeostasis, gene transcription, actin cytoskeleton remodelling and long-distance communication between cells using extracellular vesicles (EV). Here we provide evidence showing that the amino terminal of Cx43 regulates its release, and that of other cargo, into EVs. Treatments that induce mitochondrial or lysosomal damage increase the release of mitochondrial and lysosomal proteins in EVs, an effect that is exacerbated by Cx43 expression. Moreover, Cx43 expression can also induce the loading of several disease-related aggregation-prone proteins into EVs. Interestingly, mutation of a conserved LC3-interacting region on the amino-terminal of Cx43 abrogates its ability to promote the loading of content into EVs. Altogether these results suggest that connexin proteins play a role in maintaining cellular homeostasis through a pathway in which cellular content is diverted to EVs, a mechanism that is likely to be relevant in diseases associated with the accumulation of damaged or obsolete cellular material.

Health, Management and Economics

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Abstract:

The main objectives and strategy of this MAD's group can be split into three purposes: (i) to contribute to the measurable translation of knowledge into clinical applications, with a particular focus on measuring people's health state, well-being and quality of life, and the impact of healthcare on society; (ii) to contribute to a better interaction between the care provider and the patient; and (iii) to contribute to the economic evaluation of clinical studies.

Its activity encompasses four subgroups:

- Health Outcomes Measurement: aims to measure the impact of the healthcare service, diseases or interventions on the health status of an individual or populations. In this research area are include determining health gains in clinical trials or in economic evaluations, monitoring of disease management, determining the burden of a disease, and calculating individuals' preferences about health status.
- Healthcare Management & Social Impact: aims to analyze evidence-based health policy-making through comprehensive and rigorous analysis of the dynamics of health care system in Portugal, the psychological well-being in health organizations, the assessment of equity in the access to healthcare services, as well as the monitoring of audit systems.
- Econometrics & Economic Evaluation: aims to apply statistical models, analyze and understand the behavior of the economy in relation to the health of populations and the provision of health care.
- Health literacy: aims to support decision-making and the formulation of programs that enable individuals to increase their health literacy, through greater health knowledge and the development of personal skills.

IV Metabolism, Ageing & Disease (MAD) Meeting

Flash Talks

Unveiling Redox-Driven Adaptations through Metabolic Priming in NHDF

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Abstract:

Aging and lifestyle-related diseases are often associated with mitochondrial dysfunctions and redox imbalance. Thus, mitochondrial function may be a useful source of biomarkers for theragnostic [1]. To gain deeper insights into cellular and mitochondrial responses, we assessed NHDF under various metabolic conditions that alter their reliance on mitochondrial energy production.

NHDF were cultured in a 25 mM glucose medium (HG) and gradually transitioned to media with 5 mM glucose (LG) or no glucose (OX). Oxygen consumption rate (OCR) was monitored in real-time using a Resipher device, and the rates of ATP production and OCR were assessed using a Seahorse XFe96. As we have previously observed a significant increase in intracellular levels of reactive species (RS) in OX-adapted cells [2], we measured MitoSox Red fluorescence to estimate the levels of mitochondrial superoxide ($O2^{\cdot-}$) anion and examined the impact of RS on metabolic reconfiguration by exposing cells to the antioxidant N-acetyl cysteine (NAC) during metabolic priming.

When cells were first transitioned to 100% OX medium, OCR nearly doubled in 24 h. OX-adapted cells also displayed increased maximal and non-mitochondrial respirations ($p<0.001$), with almost 100% of the ATP production originating from mitochondria. Interestingly, NHDF cultured in HG and LG also exhibited a substantial percentage of mitochondria-derived ATP (~70%). Furthermore, a significant increase in mitochondrial $O2^{\cdot-}$ was observed in OX, compared to LG ($p<0.01$) and to HG ($p<0.001$). Additionally, when medium transition was performed in the presence of NAC, the increased OCR in OX was substantially lower than in its absence. The shift towards mitochondrial reliance observed when cells are exposed to OX medium seems to be dependent on RS, as demonstrated by the inhibitory effect of NAC. These findings hold promise for future research of redox agents in aging-related diseases.

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Clinical and molecular profiling of human visceral adipose tissue reveals impairment of vascular architecture and remodeling as an early hallmark of dysfunction

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Abstract:

Adipose tissue dysfunction is more related to insulin resistance than body mass index itself and an alteration in adipose tissue function is thought to underlie the shift from metabolically healthy to unhealthy obesity. Herein, we performed a clustering analysis that revealed distinct visceral adipose tissue gene expression patterns in patients with obesity at distinct stages of metabolic dysregulation. We have built a cross-sectional cohort that aims at reflecting the evolution of the metabolic sequelae of obesity with the main objective to map the sequential events that play a role in adipose tissue dysfunction from the metabolically healthy (insulin-sensitive) state to several incremental degrees of metabolic dysregulation, encompassing insulin resistance establishment, pre-diabetes, and type 2 diabetes. We found that insulin resistance is mainly marked by the downregulation of adipose tissue vasculature remodeling-associated gene expression, suggesting that processes like angiogenesis and adaptative expansion/retraction ability suffer early dysregulation. Prediabetes was characterized by compensatory growth factor-dependent signaling and increased response to hypoxia, while type 2 diabetes was associated with loss of cellular response to insulin and hypoxia and concomitant upregulation of inflammatory markers. Our findings suggest a putative sequence of dysregulation of biological processes that is not linear and has multiple distinct phases across the metabolic dysregulation process, ultimately culminating in the climax of adipose tissue dysfunction in type 2 diabetes. Several studies have addressed the transcriptomic changes in adipose tissue of patients with obesity. However, to the best of our knowledge, this is the first study unraveling the potential molecular mechanisms associated with the multi-step evolution of adipose tissue dysfunction along the metabolic sequelae of obesity.

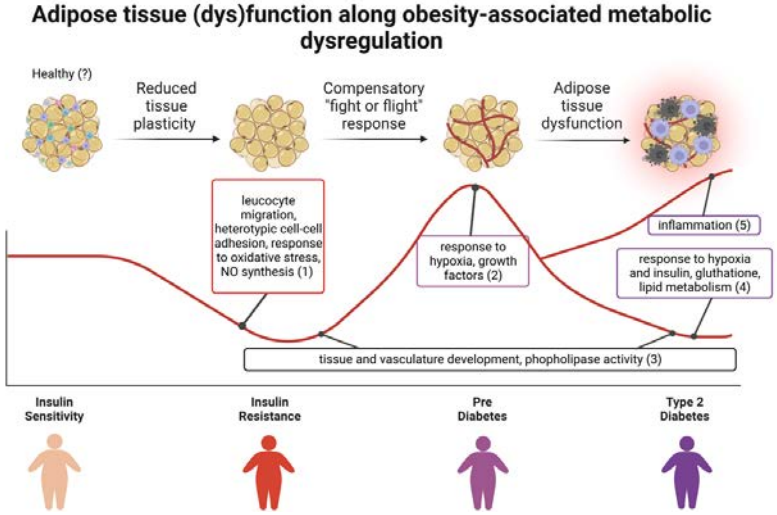

Figure 1. Schematic proposal of the putative sequential events underlying the adipose tissue (dys)function along obesity-associated metabolic dysregulation. Upon IR, a loss in overall tissue plasticity was found, with impaired vasculature development and leukocyte migration and NO synthesis, crucial for "heathy"/physiological adipose tissue remodeling. A compensatory mechanism seems to be initiated upon PD development, characterized by increased response to hypoxia, vascular adaptation and signaling through growth factors. Finally, T2D seems to be aligned with the climax of adipose tissue dysfunction, where a concomitant reduction in the response to hypoxia, insulin, glutathione and a loss in vasculature development are accompanied by an induction of inflammatory pathways.

No loss, no gain: Cholesterol efflux and sperm function deficit after capacitation in overweight and obese men

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Abstract:

Excess weight is a global epidemic that is increasing over the years affecting 50% of the adult male population. In the Reproductive Medicine Unit of CHUC, 63% of men undergoing treatments/seminal analysis present excess weight, more than expected considering the general population. Sperm samples from 1025 men were collected, together with filled medical, lifestyle and exposure questionnaires, and divided into 4 groups: normal weight (body mass index; BMI<25) with proven fertility (NW w/ PF; n=72), NW without PF (NW w/out PF; n=310), overweight (OW; 25≤BMI<30; n=451) and obese (Ob; BMI≥30; n=192). Sperm quality was assessed according to the WHO guidelines. Sperm functional markers, such as viability, chromatin/DNA status, acrosome integrity, tyrosine phosphorylation and cholesterol levels were also addressed. No differences were found between groups. However, when analysing samples before and after *in vitro* capacitation within groups, a decrease in viability and chromatin/DNA integrity was observed in the NW w/out PF, OW and Ob groups ($p<0.05$). Furthermore, OW and Ob also showed a decay in motility ($p<0.05$). Tyrosine phosphorylation, a marker of sperm capacitation, was observed to increase only in NW groups, paralleled with a decrease in acrosome integrity ($p<0.05$). To further understand the deterioration of function and capacitation in OW and Ob, levels of cholesterol were measured, as its membrane efflux is preponderant during capacitation. Once more, only the NW groups had significant cholesterol efflux during capacitation ($p<0.05$). In conclusion, capacitation is impaired in OW and Ob, followed by a deterioration in the remaining function parameters, that was also observed in the NW w/out PF group. The exact role of cholesterol during capacitation is unknown but may explain the overflow of excess weight men to reproductive clinics, along with tyrosine phosphorylation.

Can tumor educated platelets identify and predict survival of multiple myeloma patients?

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Abstract:

Tumor-educated platelets (TEPs) have emerged as a promising biosource of peripheral specific biomarkers for the detection of various cancer types. However, its role in hematological neoplasias is still poorly understood. Multiple myeloma (MM) is a genetically complex clonal plasma cell disorder, with a high biological and clinical heterogeneity. MM diagnosis and monitoring is highly dependent on bone marrow aspirate/biopsy, a procedure that apart from being invasive, does not capture MM clinical/spatial heterogeneity. Our goal was to identify a MM TEP gene expression signature and correlate it with MM prognosis and survival. Two available databases were used. The GSE183635 that includes platelet RNA-seq data of 22 MM patients and 183 healthy donors (HD); and the GSE24080 with gene expression microarrays data of CD138+ cells and survival data of 559 MM patients. All the analyses were performed using R and R Studio (version 2023.06.1) and different R packages (GEOquery, limmaVoom, edgeR, ggplot2, etc).

After rigorous bioinformatic analysis we identified 223 differentially expressed genes between platelets from MM patients and HD. Nineteen genes presented greater statistical significance with p-value <0.000001 and a fold change >2. Among these, 15 genes (*HBD*, *CA1*, *GZMH*, *IFI27*, *LY6E*, *ITGB7*, *ITGAL*, *NKG7*, *RAD23A*, *NADSYN1*, *DUSP2*, *PAXX*, *GZMB*, *TCIRG1*, and *OSBPL5*) were upregulated and 4 (*WDR11-DT*, *ZNF385D*, *GRHL1*, and *HSD17B3*) were downregulated. The prognostic value of these 223 gene MM TEP signature was further explored. Ninety-seven genes were significantly correlated with patient survival ($p<0.05$). Moreover, 11 of these genes (*EFHD2*, *CMTM6*, *ARPC5L*, *TNNC2*, *GSTP1*, *RASAL3*, *GAK*, *ANGPT1*, *DNAJC7*, *PLAAT4* and *TLR5*) presented a higher impact on MM prognosis with $p<0.001$ and hazard ratios over 1.5 (1.636±0.119).

These results demonstrated a significantly different transcriptomic profile between MM TEPs and HD platelets. Moreover, this newly identified MM TEP signature can possibly be used as a new screening test and prognostic biomarker in MM patients.

Nature's weapon against glioblastoma: unraveling the 7α-acetoxy-6β-hydroxyroyleanone (Roy) antitumor mechanism of action

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Abstract:

Glioblastoma (GB)ischaracterized by its high heterogeneityand stands as the most aggressive and prevalent glioma within the Central Nervous System. Despite considerable efforts, GB continues to exhibit a low 5-year survival rate of approximately 6%, primarily attributed to delayed diagnosis, unfavourableprognosis, and the absence of efficient therapeutic options. Consequently, there is an unmet need to develop noveland more effectivetreatments, in whichplant-based drugleadsarepromising to enhance patients' survival and well-being.In this work,we investigated the antitumor mechanism of action of 7α-acetoxy-6β-hydroxyroyleanone (Roy), a diterpene abietaneisolated from the acetonic extract ofthe South African*Plectranthus hadiensis*Schweinf. The impact of Roy'streatment was evaluated on cell death-and autophagy-related pathwaysby using a panel of glioma cell lines, namely (U87, A172,and H4).Cell death, cell cycle regulation,and mitochondrial membrane potential wereevaluated by flow cytometry. Transcripts forkey genesand proteins were assessed by qPCR andWestern blot, respectively.We show herethat Roy presents a chemotherapeutic profile against GB cells. Data analysis showed that treatment of GB cells with 16 μM of Roy leads to activation of caspase-mediated cell death, inducingapoptosis. Moreover, Roy's treatment also inhibited autophagy in GB cells, a pro-survival tumoral mechanismused by the cells to survive under hypoxia and starvation conditions, by impairing autophagy progression and by targeting AMPK and mTOR pathways.Furthermore, our results indicated that Roy likely impacts various pathways involved in the interplay between autophagy and apoptosis, by inhibiting Bcl-2 and Beclin-1 expression andbyinhibiting the formation of autophagosome, with a decrease in the expression of Atg5-Atg12 levels and an increase in theAtg5 levels.This work highlights Roy's potential as a drug lead and it is worth to develop an efficient and targeted delivery system for GB cells.

Zinc synergizes with cytarabine and olaparib increasing apoptosis and DNA damage in acute myeloblastic leukemia

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Abstract:

DNA damage response (DDR) is key for genomic stability and zinc (Zn) is a DDR cofactor. In acute myelobastic leukemia (AML), Zn decreases and DDR defects are often observed, but the role of Zn supplementation as adjuvant to AML therapy are not well understood. We evaluated the potential effects of Zn in combination with cytarabine (AraC, conventional drug) and Olaparib (Ola, DDR inhibitor) in AML models.

HEL, K-562, NB-4, and THP-1 cell lines were incubated (72h) with increasing AraC or Ola concentrations in monotherapy and combination with $ZnSO_4$ (IC_{25}). Cell density and viability were evaluated by trypan blue assay. We also analyzed the type of cell death by flow cytometry (FC), using annexin V/7-AAD staining, the expression of apoptotic markers cleaved PARP and active caspase-3 (FC), and cell morphology by optical microscopy (May Grunwald-Giemsa staining). Cell cycle distribution and proliferation were also assessed by PI/RNAse staining and BrdU incorporation using FC. Chromosomal damage (micronucleus assay), genomic damage (γH2AX expression by FC), and the expression of 18 DDR genes by qPCR were also assessed.

Zn addition to Ola and AraC decreased cell density and viability in all cell lines compared to the observed with drugs in monotherapy ($p<0.05$). The IC_{50} (48h) of each drug were considerably reduced by Zn (AraC: 5.9 to 57.6-fold; Ola: 2.7 to 8.7x, depending on the cell line). Zn combinations increased the late apoptosis/necrosis fraction ($p<0.05$) and the expression of cleaved PARP ($p<0.05$) and active caspase-3 ($p<0.0001$). Morphological analyses shown features of apoptosis. Zn altered the cytostatic profile of AraC (S) and Ola (G_2/M) in HEL and K-562 cells, inducing G_0/G_1 arrest. In general, BrdU incorporation decreased with Zn addition. Chromosomal and genomic damage increased by the combination strategies ($p<0.05$). Several DDR genes were differentially modulated by combination treatments, with *PARP1* ($p<0.05$) and *XRCC6* ($p<0.01$) genes consistently upregulated. Zn potentiated the cytotoxic, cytostatic, and genotoxic effects of AraC and Ola in four AML models, while considerably reducing their IC_{50}. These findings may translate into safer, less toxic, and more efficient therapeutic regimens in AML with Zn supplementation.

DNA-PK inhibition as new therapeutic approach in acute myeloblastic leukemia – an *in vitro* study

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Abstract:

The DNA damage response (DDR) pathway maintains genomic stability and its deregulation has been associated with the pathogenesis of acute myeloblastic leukemia (AML). The DNA-dependent protein kinase (DNA-PK) is a component of DDR and a key player in the non-homologous end joining pathway. The aim of this work was to assess the therapeutic potential of a DNA-PK inhibitor (AZD-7648) in *in vitro* models of AML. This study used seven AML cell lines of different molecular and cellular subtypes (HEL, HL-60, K-562, KG-1, LAMA-84, NB-4, and THP-1). CNVs and gene methylation were assessed by MS-MLPA and DDR gene expression and telomere length by qPCR. DSB levels and DDR kinetics were measured by flow cytometry (FC), and chromosomal damage levels by the micronucleus assay. Cells were treated with AZD-7648 and cell density and viability were analyzed for 72h using the trypan blue assay. Cell death [Annexin V/propidium iodide (PI)], cell cycle (PI/RNAse), cell proliferation (BrdU), and cleaved PARP, activated caspase-3, and DNA damage (γH2AX) levels were assessed by FC. Results were statistically analyzed with a significance level of 95%. HEL and LAMA-84 cells exhibited the highest levels of γH2AX and chromosomal damage, respectively, while KG-1 cells had the shortest telomere length. The cell lines showed differing levels of gene expression and genetic/epigenetic alterations. AZD-7648 reduced cell proliferation and viability in a dose, time, and cell line-dependent manner. HEL cells were the most sensitive with an IC_{50} of 150μM (24h), also displaying inefficient repair, whereas HL-60 and K-562 were the most resistant with an IC_{50}>200μM at all studied times. Following AZD-7648 treatment, an increase in the percentage of cells in apoptosis, a decrease in cell proliferation, and cell cycle arrest in the G_0/G_1 phase, along with elevated levels of DNA damage were observed. In conclusion, the study highlights that different AML models show variable sensitivity to DNA-PK inhibition by AZD-7648, which may be related to differences in cell DNA repair. However, these results also emphasize the need for further investigation into AZD-7648 therapeutic potential and sensitivity biomarkers.

FT08

Metalloconjugates designed to target the mitochondria of prostate cancer cells: design, synthesis and evaluation as theranostic agents

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Abstract:

The prostate-specific membrane antigen (PSMA) is a transmembrane protein markedly overexpressed in over 80% of prostate cancers. 177Lu-PSMA-617 (Pluvicto™), a radiopharmaceutical targeted at PSMA, has recently received FDA approval for the treatment of metastatic castration-resistant prostate cancer. In this complex, 177Lu is chelated by PSMA-617, a derivative of dodecane tetraacetic acid (DOTA) containing a PSMA inhibitor for selective uptake by prostate cancer cells. 177Lu shares limitations of other beta minus particle (β–) emitters, such as nephrotoxicity and resistance to β– radiation, and its replacement by Auger electron-emitting radionuclides, such as 111In, might be a safer alternative, while higher efficacy might be achieved by specifically targeting the mitochondria, regarded as highly sensitive to ionizing radiation. To this end, we synthesized two chelators, TPP-PSMA-617 and TPP-G3-PSMA-617, containing a triphenylphosphonium (TPP) group in the PSMA-617 structure. One chelator also contains a cathepsin B cleavable (triglycine; G3) linker between the PSMA inhibitor and the DOTA moiety, for the intracellular generation of smaller radioconjugates. The biological activity of the indium (111In and natIn) complexes of the novel chelators and of their single-targeted equivalents, directed at either PSMA (PSMA-617) or mitochondria (TPP-DOTA), was tested in three human prostate cell lines — two sublines of the PC-3 cancer cell line, one not expressing (PC-3 FLU) PSMA, the other overexpressing it (PC-3 PIP) — and in the PSMA-negative PNT2 cell line, derived from normal prostate tissue. All complexes exhibited low cytotoxicity against all cell lines and all those containing the PSMA-binding moiety strongly inhibited PSMA. Cell binding and internalization were substantial in PC-3 PIP cells and negligible in PC-3 FLU cells. The preliminary assessment of our novel complexes showed that they possess characteristics suitable for highly targeted therapy against prostate cancer.

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Impact of HER2+ brain-tropic breast cancer cells in blood-brain barrier dysfunction during the premetastatic niche formation

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Abstract:

Up to 50% of HER2+ breast cancer patients eventually develop brain metastasis (BM), with a median survival of less than 1 year after diagnosis. The disruption of the blood-brain barrier (BBB) by breast cancer cells (BCCs) is required for tumor cells to reach the brain. However, the pathways that drive these events remain poorly understood. Here, we aim to evaluate how primary tumors modulate the BBB permeability to facilitate the extravasation of BCCs into the brain.

A BBB in vitro model was exposed to the secretome derived from HER2+ BCCs and their brain-tropic variants. BBB integrity was assessed by measuring the transendothelial flux of a 4kDa-fluorescent dye, the TEER and the expression of tight and adherens junction proteins. Nude mice were assigned into 3 groups: the first was pretreated with BCC-derived secretome, while the second was injected orthotopically into the mammary gland to induce primary tumor formation. The third group was inoculated with brain-tropic cells intracardially to induce BM. BBB integrity was assessed in vivo by near-infrared fluorescence imaging, and ex vivo by collagen IV and albumin immunostaining in the brain.

Brain-tropic cells secrete specific bioactive factors that disrupt the BBB in vitro, facilitating their transmigration into the brain. In vivo, animals pretreated with brain-tropic cell-derived secretome exhibited structural changes in the BBB, as evidenced by a decrease in collagen IV and an increase in albumin immunoreactivity, along with the accumulation of 20kDa dextran into the brain. These BBB alterations were also observed in animals harboring a localized primary tumor without BM, confirming a systemic-mediated effect. Importantly, these changes in BBB permeability facilitated the formation of BM.

Our results emphasize the importance of BBB disruption as a crucial step in the extravasation of BCCs and formation of BM and highlight the contribution of tumor-secreted factors to this process.

Maternal Obesity disrupts offspring's cardiac aging trajectory via protein O-GlcNAcylation and stiffness with sex-dependent variations

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Abstract:

Maternal obesity (MO) incidence is rising and is related to offspring's early development of cardiovascular disease. We previously identified new early mechanisms of cardiac aging in baboons related to decreased metabolic function, upregulation of hexosamine biosynthetic pathway (HBP), and heart stiffness (PMID: 38014295). Whether MO during pregnancy impairs these aging mechanisms in offspring, with potential long-lasting impacts, remains unknown. A MO-Sprague-Dawley rat model was obtained by consumption of a high-fat/high-sugar diet (HFHS) starting 6-weeks before pregnancy. Offspring were kept under a control diet. Heart tissue from male- and female-offspring was collected at 6-, 16-, and 32-weeks-old (total controls=34; MO=32). Protein levels were quantified by immunoblotting and mitochondrial ATP concentration by luminescence. A two-way ANOVA test was performed and $p<0.05$ was set as statistically significant. Cardiac age-related stimulation of HBP was validated by increased protein O-GlcNAcylation ($p<0.01$) and increased heart stiffness by augmented levels of YAP ($p<0.01$) with smaller levels of p-YAP ($p<0.01$) in both male and female offspring. Age-associated mitochondrial ATP accumulation ($p<0.01$) in the heart suggests a general decrease in metabolism in a sex-dependent way (interaction: $p<0.01$). Autophagy, a hallmark of aging, is also downregulated with age in both sexes ($p<0.01$). MO changed offspring protein O-GlcNAcylation with aging in a sex-dependent way – increasing in males ($p<0.01$) and decreasing in females ($p=0.04$). Concomitantly, YAP levels in MO are increased in males ($p<0.01$) and decreased in females ($p=0.05$) without changes in phosphorylation levels. LC3-II/I ratio is decreased only in MO-females ($p<0.01$). We validated novel mechanisms of aging in rat hearts. MO during pregnancy impairs these mechanisms changing offspring's aging trajectory and likely increasing predisposition to cardiovascular disease.

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Technology under FCT-Post-doctoral Fellowship (SFRH/BPD/116061/2016), FCT-doctoral Fellowship (SFRH/BD/11934/2022; SFRH/BD/11924/2022; SFRH/BD/5539/2020), project grant PTDC/DTP-DES/1082/2014 (POCI-01-0145-FEDER-016657), CENTRO-01-0246-FEDER- 000010 (Multidisciplinary Institute of Ageing in Coimbra), strategic projects UIDB/04539/2020, UIDP/04539/2020, LA/P/0058/2020; UIDB/00617/2020: doi:10.54499/UIDB/00617/2020 and UIDP/00617/2020: doi: 10.54499/UIDP/00617/2020.

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And what about antidepressants: do they affect human spermatozoa?

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Abstract:

Depression currently affects about 280 million people worldwide and its prevalence has been increasing dramatically, especially among the young and people of reproductive age, which consequently leads to an increase in antidepressant (AD) consumption. In fact, Portugal is currently the OECD country ranked 2ndin AD consumption. So far, the role of ADs in male fertility has been scarcely studied. With that in mind, this studyintended to determine the possible *in vitro*mechanisms of action of fluoxetine and sertraline -the most prescribed ADs in Portugal -using concentrations previously reported for blood and seminal fluid. Spermatozoa were incubated for up to 24h at 37°C and 5% CO2and important functional parameters such as motility, viability, and mitochondrial membrane potential (n=11-13),cellular ROS production(n=15), chromatin/DNA integrity (n=20), acrosome status and tyrosine phosphorylation were assessed (n=10). Untreated controls were also used. At physiological levels, fluoxetine consistently decreased progressive motility throughout time, while promoting fluctuations in ROS levels and sperm capacitation, without affecting, however, viability, mitochondrial membrane potential, acrosome reaction nor chromatin/DNA integrity. Sertraline, on the other hand, had little to nonsignificant impact at low doses but affectedalmost all tested parameters at supraphysiological concentrations. Altogether, our results suggest that these ADs may impair human sperm function, possibly through different mechanisms of action, but only fluoxetine exhibits deleterious effects at doses found *in vivo*.

Acknowledgments: This work was financed by the European Regional Development Fund (ERDF), through the Centro 2020 Regional Operational Programme under the project CENTRO-01-0145-FEDER-000012 (HealthyAging2020), and the COMPETE 2020 -Operational Programme for Competitiveness and Portuguese national funds via FCT –Fundação para a Ciência e a Tecnologia, under the projects UIDB/04539/2020, UIDP/04539/2020 and LA/P/0058/2020, and the individual research grant 2020.06698.BD, and through the L'OHMI_DHM-E/2019/Proj.3 project.

Postnatal overfeeding triggers a neurodevelopmental delay and induces anxious-like behaviour accompanied by sex-and brain-region-specific synaptic and metabolic changes

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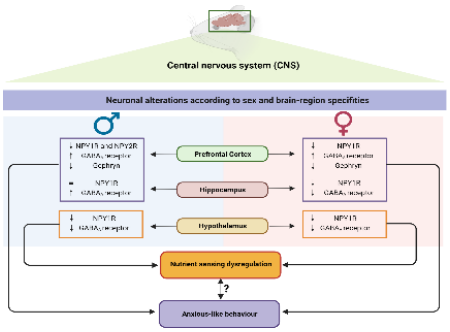
Abstract:

The first periods of life are crucial to modulate offspring neurodevelopment and behaviour. Nutritional disturbances during the postnatal period can have long-lasting effects and lead to emotional and cognitive impairments in later life. Despite neuronal connections disruption can contribute to social and behaviour alterations, dysregulation of neuroendocrine pathways involved in nutrient-sensing balance, may also cause such impairments, although the underlying mechanisms are still unclear. We aimed to evaluate sex-specific neurodevelopmental and behavioural changes upon postnatal overfeeding and dissect the potential underpinning mechanisms at central nervous system level, with a focus on the interconnection between synaptic and neuroendocrine molecular alterations. At postnatal day 3 litters were culled to 3 animals (small litter procedure). Neurodevelopmental tests were conducted at infancy, whereas behavioural tests to assess locomotion, anxiety, and memory were performed at adolescence, together with molecular analysis of the hippocampus, hypothalamus, and prefrontal cortex. At infancy, females presented impaired acquisition of auditory response, eye-opening, olfactory discrimination, and impaired vestibular system development, suggesting that female offspring neurodevelopment/maturation is deeply affected. Male offspring presented a transitory delay in locomotor performance, while both offspring had fewer upper limbs strength. At adolescence, both sexes presented an anxious-like behaviour without alterations in short-term memory retention. Both males and females presented lower NPY1R levels in a region-specific manner. Furthermore, both sexes presented synaptic changes in the hippocampus (lower $GABA_A$ in females and higher $GABA_A$ levels in males), while in the prefrontal cortex, similar higher $GABA_A$ receptor levels were observed. Thus, we point out the role of NPY as a potential bridge between energy balance and emotional behaviour, demonstrating that both offspring have brain-region-specific alterations in this system which contributes to metabolic and behavioural disruptions later in life.


Central nervous system (CNS)
Neuronal alterations according to sex and brain-region specificities
Prefrontal Cortex
Hippocampus
Hypothalamus
Nutrient sensing dysregulation
?
Anxious-like behaviour

IV Metabolism, Ageing & Disease (MAD) Meeting

Poster

Role of mitochondrial-thiol homeostasis in neurodegeneration: implication for neurodegenerative diseases

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Abstract:

Neurodegenerative diseases (NDs) are linked with oxidative stress, mitochondrial dysfunction, and neuronal death. Interestingly, mitochondria have an independent antioxidant defence that may constitute the first line of cell defence and thus may have an important role protecting the cells against free radicals. In this sense, the aim of this work was to understand whether the disruption of the mitochondrial antioxidant system could be a common mechanism causative of neuronal death. To that, two different cellular models of NDs were exposed to a derivate of 1,5-dichloro-2,4-dinitrobenzene (CDNB), that depletes glutathione and inhibits key thiol redox enzymes, specifically modified to accumulate in mitochondria (MitoCDNB). Parameters such as cytotoxicity, ATP levels, ROS production, mitochondrial activity (including mitochondrial membrane potential, oxygen consumption rate (OCR)), and overall protein oxidation were evaluated. To address if the disruption of the mitochondrial thiol system can be targeted for therapy, a mitochondria-specific hydrogen sulfide donor (AP39) was also tested.

Briefly, a similar response was observed for both cell models used, with low concentrations of MitoCDNB (1-10 μM) resulting in some disturbance of mitochondrial activity, without causing cell death which is only achieved with higher concentrations of the compound (above 25 μM). MitoCDNB stimulation resulted also in a gradual decrease in OCR, and in a noticeable positive correlation between MitoCDNB concentrations and ROS production, as well as overall protein oxidation. AP39 appears to revert MitoCDNB's effect in ROS production, but not in the metabolic activity.

These preliminary results already point out for a link between mitochondrial thiol homeostasis dysregulation, mitochondrial dysfunction, and consequent neuronal death. Further assays are required to support this observation and to clarify the exact mechanism and players involved in this process.

P02

The impact of impaired peroxisomal β-oxidation on fatty acid metabolism under lipotoxicity insults

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Abstract:

Peroxisome and mitochondria are subcellular organelles involved in fatty acid degradation, contributing to reactive oxygen species (ROS) detoxification. Importantly, peroxisomal impairment is likely to induce mitochondrial dysfunction and vice versa, but the impact of these complex processes on disease pathology and progression is still poorly understood. This is more evident in nonalcoholic fatty liver disease (NAFLD), one of the most common chronic liver diseases. Peroxisomal acyl-coenzyme A oxidase 1 (ACOX1) is the first and the rate-limiting step in peroxisomal β-oxidation and the main H_2O_2 producer within the peroxisome. Here, we aim to investigate whether knockout and/or inhibition of ACOX1 impacts cell viability, neutral lipids accumulation and mitochondrial polarization under lipotoxic insult.

Human hepatoma-like cells (HepG2 WT and HepG2 Acox1$^{-/-}$) were incubated with supraphysiological concentrations of palmitic acid (C16:0) or hexacosanoic acid (C26:0) to target mitochondrial and peroxisomal β-oxidation, respectively. In addition, thioridazine (TDZ) was used to specifically inhibit peroxisomal β-oxidation. We observed that C16:0 dose dependently reduced cell viability of HepG2 and these effects were more noticed in WT cells. By contrast, C26:0 did not affect cell viability of HepG2 WT or HepG2 Acox1$^{-/-}$at all tested concentrations. Moreover, we confirmed that HepG2 WT treated with C16:0 trends to accumulate more lipid droplets than HepG2 Acox1$^{-/-}$. Interestingly, TDZ prevented lipid droplet accumulation in HepG2 WT and HepG2 Acox1$^{-/-}$ cells, the effects being more noticeable in HepG2 WT cells. The results also show that C26:0 treatment does not increase lipid droplet accumulation in both cell lines. Our preliminary data suggests that modulation of peroxisomal β-oxidation impacts lipid droplet accumulation although more studies are required to understand the impact on mitochondrial metabolism.

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Role of Lysosome-Mitochondria Membrane Contact Sites in Nutrient Sensing

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Abstract:

Ageing is defined by a set of physiological alterations leading to organismal decay. One of the ageing markers is metabolic dysfunction, which is commonly accompanied by lysosomal and mitochondrial decline. Recent studies show that these two organelles communicate with each other through membrane contact sites (MCS) to regulate fission of mitochondria, as well as to transport calcium, iron, and cholesterol between them. Nutrient sensing is important for the coordination of cellular metabolism, and it is known that both organelles are key players in sensing nutrients, such as lipids, amino acids, and glucose. However, if and how lysosome-mitochondria MCS can regulate these mechanisms is unknown.

We optimized and applied a split-GFP-based contact site sensor (SPLICS) to visualize and quantify lysosome-mitochondria contacts in different nutrient sensing conditions. Using mouse embryonic fibroblasts as a model, we observed that manipulating different types of nutrients in cells impacts the number of lysosome-mitochondria MCS and identified the regulatory hub that coordinates these contacts with cellular metabolism. Future studies will aim to look at these effects in senescence models, helping us uncover how lysosome-mitochondria MCS dynamics and functions contribute to the process of ageing and ageing-related diseases.

Effects of Cold Atmospheric Plasma in Breast Cancer Stem Cells

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Abstract:

Breast cancer has the highest number of new cases and the second-highest mortality rate in the world for both sexes and all ages. Current treatments for this disease include surgery, chemotherapy, radiotherapy, and hormonal therapies, which are effective but also have limitations and side effects. Cold Atmospheric Plasma (CAP) has emerged as a potential treatment, exhibiting selectivity to cancer cells while preserving healthy cells. Cancer stem cells (CSCs) play a critical role in tumour recurrence and metastasis due to their heightened self-renewal and invasion among the cancer population. The main goal of this study was to assess the effects of CAP on breast CSCs. These studies were performed in two breast cell lines representing triple negative (HCC1806) and hormone-dependent (MCF-7). The evaluation included plasma cytotoxicity through metabolic activity, sphere formation capacity, self-renewal, and projection area regarding different exposure times. Metabolic activity significantly reduced after 60 seconds (71.02±5.56%, p=0.0137) of plasma treatment, most marked after 240 seconds (23.64±5.65%, p=0.0054) in the MCF-7 cell line. HCC1806 significantly decreased after 240 seconds (39.59±5.45%, p=0.0081). Sphere formation capacity and self-renewal decreased with increasing exposure to cold plasma in stem cells from both lines after 120 and 240 seconds. The projection area of the spheres decreased in HCC1806 after 240 seconds from 3686±319.3 pixels to 419.3±242.1 pixels, p=0.0179; however, it increased in MCF-7 after 120 seconds of exposure. Plasma demonstrated therapeutic potential in targeting breast CSCs, encouraging future studies.

Acknowledgment: PhD studentship grant by the Portuguese National Funding Agency for Science and Technology (FCT), 2022.12228.BD. Institutional funding from FCT strategic projects (UIDB/04539/2020, UIDP/04539/2020) and by COMPETE-FEDER (POCI-01-0145-FEDER-007440).

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P05

Steroidal oximes as a potential new therapeutic for different types of cancer

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Abstract:

Cancer remains one of the most life-threatening diseases worldwide despite the number of therapies currently available. Both steroids and oximes have been associated with antitumor activity. Thus, we decided to design and synthesize new steroidal oximes and evaluated their potential antitumor activity against lung and prostate cancer cells, aiming to find alternative cytotoxic compounds for cancer treatment.

Compounds **OX1**, **OX2** and **EP2OX** and their parent compounds **P1**, **OL2** and **EP2**, were synthesized, and their cytotoxicity evaluated in H1299 and PC3 cell lines through SRB assay after the treatment with the compounds (1-75 μM). Cell viability, cell death profile, alterations on cell cycle and mitochondrial membrane potential were assessed by flow cytometry.

OX1 and **EP2OX** decreased both cell proliferation in a dose-dependent manner but **OX2** did not have the same effect. Overall, the parent compounds decreased cell proliferation in a less pronounced way, proving that the introduction of an oxime group was beneficial for the cytotoxicity displayed. Moreover, the best compound was **EP2OX** in both cell lines with IC_{50} values of 1.1 and 2.02 μM in H1299 and PC3, respectively. **OX1** was also quite active with IC_{50} values of 18.69 μM (H1299) and 29.95 μM (PC3). Flow cytometry studies showed that **EP2OX** was able to decrease cell viability in H1299 and PC3 cells by causing apoptosis and/or necrosis, which was accompanied by a blockage at phases G2/M and S, depending on the cell line and concentration. Considering the mitochondrial membrane potential, **EP2OX** induced mitochondrial dysfunction.

Our results show that **EP2OX** possesses a beneficial antitumor effect from the introduction of the oxime group, which is mediated by apoptosis/necrosis. This effect encourages further studies on its mechanism of action and selectivity in order to discover new molecules for cancer treatment.

Exploring New Horizons: Synergistic PARP Inhibitor Combinations for a Revolutionary Approach to Pancreatic Cancer

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Abstract:

Pancreatic cancer (PanC), with a 5-year survival rate of approximately 5%, is among the most lethal cancers globally needing novel and more effective treatment approaches. Poly(ADP-ribose) Polymerase inhibitors (PARPi) obstruct the ability of cancer cells to repair their damaged DNA. As a result, the combination of PARPi and DNA-damaging agents (DNA-DA) or external beam radiation therapy can escalate the quantity of DNA damage beyond the repair mechanisms, enhancing treatment effectiveness while minimizing adverse effects. This study aimed to investigate the potential of combining PARPi with DNA-DA or radiotherapy to improve PanC treatment outcomes.

Olaparib (OLA), a PARPi, was combined with DNA-DA (irinotecan or oxaliplatin) in the MIA PaCa-2 cell line. The drugs were combined at a constant ratio based on their IC_{50}. Three combination regimens were tested: simultaneous administration, OLA administered 24 hours before or after each DNA-DA. The combinations were evaluated 24, 48, and 72 hours after treatment using the SRB assay. The Chou-Talalay approach was used to assess synergism. OLA was also combined with radiotherapy at a fixed non-cytotoxic concentration (1μM) to evaluate radiosensitization using the clonogenic assay. The sensitization enhancement ratio (SER) was used to quantify the radiosensitization effect.

OLA combined with DNA-DA, especially irinotecan, showed a synergistic effect, that was more pronounced when OLA was administered 24 hours after the irinotecan. A slight synergistic effect was observed with OLA administered 24 hours after oxaliplatin. Additionally, OLA demonstrated a radiosensitizing effect in the MIA PaCa-2 cell line with a SER=1.39.

This study demonstrates the potential of combining OLA with irinotecan or oxaliplatin (in a sequential regimen) and highlights OLA's radiosensitizing effect as a future therapeutic approach for PanC. The induction of single-strand breaks by the DNA-DA or radiotherapy with the consequent inhibition of their repair by OLA may help explain the synergistic and radiosensitizing effects observed. Further investigation is warranted to clarify such mechanisms..

Characterization of a human amniotic membrane extract and its fractions: a proteomic study

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Abstract:

Human amniotic membrane (hAM) presents anti-tumor properties, such as anti-angiogenic and pro-apoptotic activity. We previously showed that total hAM extract (hAME) and its fractions obtained by solubility fractionation leads to decreased viability on hepatocellular carcinoma (HCC) cells. Thus, we aimed to characterize the hAME and fractions proteomic profile.

Fractionation of hAME was performed through ammonium sulphate (AS) precipitation by sequentially adding 10, 25 and 50% AS to hAME, on ice, 15min, centrifuged at 14000 G, 15min. Soluble fractions (10S/25S/50S) were submitted to salting out with PBS by centrifugation (4000G, 60 min, VivaSpin® tubes, 30kDa cutoff). SDS-PAGE electrophoresis was performed with 30µg of protein of hAME, 10S, 25S and 50S in a 12.5% acrylamide gel, resolved at 150V. Gel was stained with 0.12% commassie blue G-250 solution. In gel digestion was performed and identification of proteomic profile was determined by mass spectrometry (MS) and subsequent bioinformatics analyses.

We identified 1295 proteins with at least 2 peptides by analysis of whole gel lanes by MS. PCA analysis showed a clustering of proteomic data from hAME and 50S fraction, and no differences on proteomic data from 10S and 25S fractions. The heatmap represents the 50 most abundant proteins and relative abundance among hAME, 10S, 25S and 50S samples. These proteins cover a wide range of cellular functions. Comparing relative abundance between hAME and fractions, we identified 16 proteins at 10S, 5 proteins at 25S and 355 proteins at 50S with lower relative abundance. There was 1 protein with higher relative abundance at fractions 10S and 50S comparing to hAME.

We successfully determine the proteomic profile of hAME and fractions. Lower relative abundance of proteins in fractions are due to lower protein solubility in ammonium sulphate.

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Photodynamic therapy based on ring-fused chlorins: a promising strategy to eliminate endometrial cancer stem cells?

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Abstract:

Endometrial cancer (EC) conservative treatment has become challenging due to the potential risk of recurrence, evidencing the urgency to develop new therapies. The recurrence can be associated with cancer stem cells (CSC), recognized as responsible for tumorigenesis and resistance to therapy. Thus, we explored photodynamic therapy based on ring-fused chlorins (Px-PDT) as a new conservative therapy to EC, eliminating CSC. ECC-1 and RL95-2 were incubated with 0.1-1 μMof Px1 (dihydroxymethyl), Px3 (dicarboxylic acid), and Px4 (dimethylesther) chlorins, for 24 hours, followed by a light activation (7.5mW/cm2, 10J). Viability, types of cell death,cell cycle, and relevant ROSwere evaluated by flow cytometry and an indirect measurement using ROS scavengers. The efficacy of Px-PDT wasevaluated in CSC, isolated withasphere-forming protocol, by resazurin assay.In parallel, the subcellular localization of Px1 was evaluated through confocal microscopy. Px-PDTdecreased CSC metabolic activity, presenting a concentration-dependent behaviour. Likewise, Px-PDT decreased EC cellsviability, mainly inducing death by late apoptosis/necrosis. Regarding the cell cycle, Px-PDTinduced an increase in the subG0/G1andG0/G1 phases, witha decrease in the S phase. ROS assessmentindicatedan important role of singlet oxygenand an imbalance of peroxides and superoxide anion. Px1 accumulates into cytoplasmand organelles, mitochondria and endoplasmic reticulum, and plasma membrane, without colocalization in the nucleus. Concerning CSC, we proved that Px1 presents the ability tointernalize uniformly into spheres.Px-PDT seems to be a promising therapy for EC,encouraging anendometrial CSC-targeted PDT strategy.

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Cellular response of human skin keratinocytes to external beam radiotherapy

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Abstract:

Knowledge of the biological effects of external beam radiotherapy (ERT) exposures on the skin is essential to understanding related secondary skin lesions. Therefore, we intend to evaluate the biological effects of ERT induced by IR in human skin keratinocytes.

A human skin keratinocytes cell line (HaCaT) were exposed to therapeutic doses of X-rays (0-10 Gy) within a proper phantom ($0.5x10^6$cels/mL), using a TrueBeam linear accelerator of 6MV energy. Thus, clonogenic assay were done to access radiosensitibility and to obtain mean lethal dose (DL50). Flow cytometry was used for the evaluation of biological effects induced by ERT on cell viability (annexin V/propidium iodide) and cell cycle (propidium iodide/RNase) as well as genotoxicity by micronucleus assay, 7 days post-irradiation. Cell migration (scratch assay) was evaluated till 6 days post-irradiation. The exposition of HaCaT cells to 0-10 Gy of X-rays lead to a decrease in cell survival, adjusted to a linear quadratic model, with a LD50=3.64 Gy (CI95% 3.49-3.80 Gy) and a SF(2Gy) of 0.82 (CI95% 0.78-0.86). The irradiation with 2Gy or LD50 dose (4Gy) induced a decrease in cell viability to 78,25 ± 3,73% ($p<0,05$) and 66,00 ± 11,74% ($p<0,001$), respectively. This is associated with an increase in cell death by initial apoptosis and also an arrest in S and G2/M phases and a decrease in cell migration, after irradiation with LD50 or higher doses. The results showed an increase in genotoxicity, even after exposure to 2Gy.

Exposure of human keratinocytes to therapeutic doses of EBR induces long-term radiobiological effects and genotoxicity, promoting cell death. Moreover, the decrease in cell migration, even at 2Gy, could be correlated with the severity of skin injuries, and this should be considered during ERT planning.

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P10

In vitro characterization of dedifferentiated gingival fibro-blasts

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Abstract:

Regenerative dentistry (RD) uses a combination of stem cells, scaffolds and microenvironment stimulus to regenerate the loss of damaged tissues, surpassing the limitations of conventional treatments using synthetic materials. The availability of stem cells is a major limitation for RD procedures, so new sources are fundamental. Since gingival fibroblasts (GFs) are easy to collect and expand from patients, this work aims to use GFs to obtain stem-like cells.

Mouse GFs were dedifferentiated using reversine. The expression of key markers CD11b, CD45, CD90, CD105, CD106, and telomerase reverse transcriptase (TERT), was evaluated by flow cytometry. The alkaline phosphatase (ALP) gene expression was determined by RT-PCR, and the protein expression by live immunofluorescence. Oct-3/4 and Nanog protein expression was evaluated by western blot and the DNA methylation using an ELISA kit. Following dedifferentiation, osteogenic, adipogenic, and chondrogenic differentiation were induced using specific media and were evaluated through staining and subsequent quantification.

The obtained stem-like cells present a significantly increased expression of mesenchymal stem cell markers CD90, CD145 and CD106, while CD11b and CD45 expression remained undetectable. No changes were detected in ALP gene and protein expression, Nanog protein expression, and DNA methylation; however, a significant increase in TERT expression was seen. Dedifferentiated cells successfully underwent osteogenic, adipogenic, and chondrogenic differentiation.

GFs were successfully dedifferentiated into stem-like cells with trilineage differentiation potential. The dedifferentiation process induced genetic and functional changes, enhancing the stem cell properties of the cells. .

19 years of rare SinEnergies: Science, Communication, Awareness, Fundraising

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Abstract:

The LBioMiT team carries out translational research and diagnosis in rare mitochondrial diseases. In its commitment to society and its mission to raise awareness for research into these group of disorders, the team developed several science communication initiatives, including lectures, concerts, exhibitions and colloquiums, which raised over €60,000 for research. Some initiatives were granted funding: UC730 prizes (2020) and EEA Grants (2022). The impact of these activities is maximized through partnerships with several companies and institutions, such as Rei dos Leitões, Orquestra Clássica do Centro, Coimbra Music Conservatory, Cooperativa Agrícola do Távora, Court of Appeal of Coimbra, PPL Crowdfunding, among many others.

In the past decade (2014-2023), LBioMiT has had over 600 pieces of news media, which amounted to >€3.5 million automatic advertisement value and an average circulation (per news piece) of 25,000.

We believe that this is part of our mission as scientists, contributing to health literacy and bringing patients and caregivers closer to current research. We hope in the future to construct an impact evaluation kit to understand current approaches and improve them.

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Beating For Two: unraveling maternal cardiac redox changes that remain 8-weeks postpartum

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Abstract:

Pregnancy can shape future maternal health, occurring multiple cardiovascular adaptations to sustain optimal fetal development. Despite the knowledge on pregnancy-related physiological cardiovascular alterations, the molecular adaptations remain unexplored, particularly in the postpartum period. We aimed to grasp the maternal cardiac molecular traits induced by uncomplicated pregnancy that persist 8 weeks postpartum.

Female Sprague-Dawley rats (n≥6) were fed a chow diet during pregnancy and after delivery. Non-pregnant counterparts received the same diet. The animals were euthanized 8 weeks postpartum with 25 weeks of age. Data were analyzed using Mann-Whitney or t-student test according to Gaussian distribution, $p<0.05$ statistically significant.

Activated protein AMP-dependent kinase (AMPK) was increased in the hearts of rat mothers ($p=0.03$) as well as its ratio to total AMPK ($p=0.04$) at 8 weeks postpartum. Hypoxia-inducible factor-α (HIF1α) transcript level was also increased ($p=0.05$). Cardiac nuclear factor erythroid 2–related factor 2 (NRF2) higher levels ($p=0.01$) were accompanied by increased levels of glutathione peroxidase-4 ($p=0.01$) and catalase activity ($p=0.05$). Moreover, maternal cardiac mitochondria showed increased levels of ATP and NAD(P)H ($p=0.03$ and $p=0.02$, respectively).

Molecular adaptations, marked by an unbalanced redox state, were observed in the maternal hearts 8 weeks after uncomplicated pregnancies, supporting the postpartum period as part of the critical window for future maternal cardiovascular health assessment.

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The role of micronutrients of invariant Natural Killer T cells and their function in colon cancer

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Abstract:

Invariant natural killer T cells (iNKT) are innate-like T cells that recognize lipid antigens and play critical functions in tumor immunosurveillance by initiating a cascade of immune reactions to eliminate transformed cells in early stage, as in colon cancer, the third-deadliest cancer worldwide.

Preliminary data suggest that abrogation of retinoic acid (RA) signaling in thymocytes leads to iNKT cell development disruption and increased colitis-associated tumor malignancy, suggesting a crucial role for RA in iNKT cells development and in colon cancer immunosurveillance.

This proposal aims to determine the role of diet-derived retinols in iNKT cell development and its function in colon cancer. For that, we will use cellular and molecular state-of-the-art tools, as tissue-specific genetic models and RNA sequencing in combination with intestinal cancer model. This study will uncover a new metabolite-dependent pathway in iNKT cell development and cancer, shedding light on dietary-regulated non- conventional T cell role in tumor microenvironment.

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Searching for cosmetic ingredients in strawberry-tree leaves' and turpentine-tree fruits' extracts

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Abstract:

Fruits of turpentine-tree (*Pistacia terebinthus* L.) and leaves of strawberry-tree (*Arbutus unedo* L.) have been used in traditional medicine for the treatment of skin conditions such as psoriasis, wounds, and infections. Bearing in mind these traditional uses, we evaluated here their potential as natural ingredients for cosmetics. For that, the hydroalcoholic extracts (EtOH 80%) were prepared and chemically characterized by FTIR-ATR and by HPLC-ESI-MS^n. Total phenolic compounds and total flavonoids were also determined by spectrometric methods. The cytotoxicity of these extracts was determined *in vitro* using Normal Human Dermal Fibroblasts (NHDF), evaluating both the cellular metabolic activity by Alamar Blue® assay and cell mass by the sulforhodamine B assay. Afterward, we evaluated the ability of the extracts to protect NHDF from the oxidative toxicity evoked by 0.5 mM *t*-BHP (*tert*-butyl hydroperoxide). Noteworthy, skin irritation was evaluated using the SkinEthic™ Reconstructed Human Epidermis (RHE) model, in compliance with the OECD Test Guideline No. 439. The viability assays showed non-toxic concentrations up to 0.4 and 0.2 mg/mL of *P. terebinthus* and *A. unedo* extracts, respectively. When cells were challenged with the pro-oxidant *t*-BHP, a larger protection of cells metabolic activity ($^{****}p<0.0001$) was achieved for the *P. terebinthus* extract at 0.4 mg/mL. Furthermore, the irritation test demonstrated that our extracts are not irritant for the epidermal layer of the skin, thus confirming their safety profile. Comparing the two studied extracts, the extract from turpentine-tree fruits bears promising natural ingredients with a high potential to be included in the future development of cosmetics.

Metabolic Remodelling in Adipose Tissue: Insights from Roux-en-Y Gastric Bypass Surgery on Fructose Production

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Abstract:

Consumption of a hypercaloric diet rich in glucose and fructose is a risk factor for developing Type 2 Diabetes (T2D). Hyperglycaemia is estimated to activate the endogenous fructose synthesis via the polyol pathway (POP) in many tissues, contributing to the fructose burden. Even though POP activation has been associated with Diabetes comorbidities, its impact in the adipose tissue is unclear. Our goal was to assess gene expression of fructose metabolism and POP enzymes in the adipose tissue of subjects with and without T2D. We used a cohort undergoing Roux-en-Y gastric bypass (RYGB) surgery, showing metabolic improvements and T2D remission alongside weight loss.

Subjects were recruited at the outpatient clinic of Uppsala University and the study design and clinical characterization were previously published (Almby et al., 2021; Katsogiannos et al., 2019, 2020). In brief, subcutaneous adipose tissue needle biopsies were collected at baseline and 4-, 24-, and 104-weeks post-surgery. In addition, samples were obtained from subjects without T2D (age- and BMI-matched subjects at 104 weeks post-RYGB) that did not undergo weight-loss surgery (controls). The obtained adipose tissue was analysed for gene expression of key factors involved in fructose metabolism by transcriptomics.

Aldose reductase gene expression significantly decreased at 104 weeks after surgery, compared to baseline (9.5±0.2 FPKM vs 11.8±0.5 FPKM, p=0.02), reaching levels below those observed in healthy controls (12.1±1.1 FPKM, p=0.05). The gene expression of glucose 6-phosphate dehydrogenase and 6-phosphogluconolactonase, which are enzymes of the oxidative branch of the pentose phosphate pathway, significantly decreased (11.9±0.4 FPKM vs 14.4±0.5 FPKM, p=0.02; 16.3±0.6 FPKM vs 18.4±0.9 FPKM, p=0.04). Furthermore, glucose 6-phosphate dehydrogenase expression was significantly lower at 104 weeks compared to healthy controls (14.6±0.7, p=0.004).

The observed results indicate that RYGB surgery caused a significant downregulation of the polyol pathway associated with a shift in the pentose phosphate pathway away from its oxidative mode. Altogether, these findings indicate that RYGB surgery not only contributes to T2D remission and weight loss but also influences key enzymatic processes associated with fructose and polyol metabolism in adipose tissue.

Exploring the lipidomic profiles of mice extrahepatic tissues in high-fat-induced NAFLD using 1H-NMR

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Abstract:

One of the most prevalent hepatic disorders in Western adults is non-alcoholic fatty liver disease (NAFLD), which has been surging in recent years1. In 2018, about 25% of the world's population was afflicted by NAFLD1. A major driver that contributes to NAFLD is the consumption of food that are highly processed, energy-dense, and rich in fat and sugar, which are typical of sedentary lifestyles. The impact of NAFLD on organs outside of the liver has been often disregarded, and tissues such as the heart, kidney, or skeletal muscle can reveal crucial metabolic cues on the effect of the disease on the remaining organism.

The objective of this work is to better understand the events occurring in extrahepatic tissues in a diet-induced NAFLD context, evaluating the power of using metabolomics and lipidomics to unravel interactions and changes related to the development of the disorder.

In this experiment, 24 10-week-old male C57BL6J mice were fed high-fat chow or standard chow for 18 weeks to induce NAFLD. Heart, kidney, and skeletal muscle organic extracts were processed by a 1H-Nuclear Magnetic Resonance spectroscopy (1H-NMR), followed by multivariate and univariate analysis statistical approaches.

The models created in the multivariate analysis show a clear distinction between both diets in the kidney, and in part in the skeletal muscle as well, with the heart models presenting weak performance. This could be confirmed by the univariate graphs, with more significant differences found in the skeletal muscle and kidney, and just 2 in the heart (originating from the general increase in linoleic acid).

This data is on-going work that combines with previous results in aqueous extract metabolomics of the same animals to achieve a greater picture on the development of NAFLD throughout other key organs in the organisms that play a key in the development of the metabolic syndrome.

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Promising effects of mitochondriotropic antioxidant AntiOxCIN4 on cardiac metabolism in Western Diet-induced metabolic syndrome

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Abstract:

Nonalcoholic fatty liver disease (NAFLD) is a multi-factorial disease, involving excessive hepatic lipid accumulation, oxidative stress, inflammation and mitochondrial dysfunction, in which symptomatic manifestations are often associated with cardiovascular diseases (CVDs). We previously demonstrated that mitochondriotropic antioxidant AntiOxCIN4 improved liver steatosis in Western diet-fed mice by preventing lipid accumulation due to upregulation of fatty acid oxidation, quality control mechanism and antioxidant defense system. We hypothesize that AntiOxCIN4 can also improve NAFLD/NASH-associated extra-hepatic CVDs complications.

AntiOxCIN4 (2.5mg/day/animal) was orally administrated to male C57BL/6J mice fed with a high-fat (30%), high-sucrose (30%) (HFHS) diet. Histological analysis of cardiac tissue showed that neither HFHS diet nor AntiOxCIN4 did not induce alterations in structural or inflammatory biomarkers. Conversely, proteomic data showed that HFHS diet increased the expression levels of proteins associated with mitochondrial dysfunction, gluconeogenesis and glycolysis, while decreased protein levels related with oxidative phosphorylation and estrogen receptor signaling pathway. Interestingly, AntiOxCIN4 prevented the HFHS diet-induced alterations in mitochondrial dysfunction proteins and estrogen receptor signaling pathway.

Our results suggest that the HFHS diet primarily induced cardiac metabolic alterations rather than structural changes.

The effect of free or nanocurcumin in a model of sporadic Alzheimer Diseases in mice

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Abstract:

Curcumin (Cur) is a natural compound, with diverse biological functions, including neuroprotection[1], however Cur low bioavailability associated with its low gastrointestinal absorption and high systemic clearance[2], has preclude its use at a clinical level. The use of nanotechnology strategies have emerged as a solution to overcome these limitations[3]. Therefore, the aim of this study was to investigate a formulation of Eudragit (EUD) polymeric nanocapsules (NC) loaded with Cur in a mouse model of Alzheimer's Disease (AD) induced by Streptozotocin (STZ). The animals were divided into 5 groups: Group I (control); Group II (Cur EUD Nc 0.6 mg/ml); Group III (Cur 0.6 mg/ml); Group IV (Donepezil) and Group V (STZ). The animals received the induction with STZ (3mg/kg, 3µL) or saline 0.9% (5 ml/kg, vehicle of STZ) by intracerebroventricular administration on days 1 and 3 of the protocol. The Y-maze behavioral test was performed on day 19, and the treatments were initiated on day 22 and performed in alternated days until the end of the protocol (day 37) by intragastric route. On day 34 the same behavior test was performed. When compared to the control group, the STZ-treated animals, both male and female mice, demonstrate a decrease in the spontaneous alternation behavior on near 57% and 61%, respectively, without changing the number of arms entries. The treatment with free Cur and Nc Cur EUD restored the spontaneous alternation behavior caused by STZ. Altogether, these results demonstrate that the treatment with free Cur or Nc Cur EUD was capable of restoring the damage caused by STZ in mice in terms of visual-spatial working/working memory. Future experiments aim clarify the possible mechanisms of action involved on Nc Cur EUD and free Cur will be performed, including evaluation of oxidative stress and inflammatory parameters.

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Effects of mitochondriotropic antioxidant AntiOxCIN$_4$ on mitochondrial metabolism of adipose tissue in a mouse model for Western Diet-induced metabolic syndrome

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Abstract:

Nonalcoholic fatty liver disease (NAFLD) is growing worldwide with a prevalence of about 25% in Western countries. The pathogenic importance of obesity-induced "adipose tissue failure", resulting in decreased adipose tissue functionality has been described to play an important role in the disease development and progression. Targeting oxidative stress and mitochondrial dysfunction with mitochondriotropic antioxidants can ameliorate NAFLD phenotype and associated organ metabolic failure. We previously developed a mitochondriotropic antioxidant (AntiOxCIN$_4$) that improved liver steatosis in high fat, high sugar (HFHS)-fed mice by preventing lipid accumulation due to upregulation of fatty acid oxidation, quality control mechanism and antioxidant defense systems. Here, we aim to investigate the effects of AntiOxCIN$_4$ on mesenchymal adipose tissue of HFHS-fed mice. AntiOxCIN4 (2.5mg/day/animal) was orally administrated for 18 weeks to male C57BL/6J mice fed with a high-fat (30%), high-sucrose (30%) (HFHS) diet for 16 weeks.

Preliminary analysis of mesenchymal adipose tissue showed that neither HFHS diet nor AntiOxCIN4 did not induce alterations in the content of mitochondrial markers or oxidative phosphorylation proteins. Similarly, no alterations were observed in oxidative stress markers. Our results suggests that HFHS diet-based intervention did not impact on mitochondrial metabolism and oxidative stress of mesenchymal adipose tissue. Although the preliminary results do not reinforce the adipocentric perspective of NAFLD more studies in adipose tissue inflammation are necessary.

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Brain stiffness alterations in Metabolic syndromephenotype: A comprehensive exploration of metabolism and mechanotransduction crosstalk

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Abstract:

Metabolic syndrome (MetS) is a major public health concern that affects 25% of world's population.MetS encompasses several disorders including dyslipidemia, obesity, and hyperglycemia/insulin resistance, all risk factors for neurodegenerative diseases (ND) including Alzheimer's and dementia. The mechanisms underlying MetS-driven ND pathophysiology are not fully understood. Although the impairment of brain metabolic pathways impacts on tissue mechanics and vice-versa, the precise contribution of processes on disease pathology and progression remains poorly understood.Our study aims to investigate the role of mechanotransduction and metabolic dysfunction as initiators and/or potentiators of MetS to establish a potential cause-effect relationship between brain stiffness changes and brain metabolic alterations.

Hence, we fed C57BL/6J mice with a standard diet (SD) versus Western diet (WD) for 16 weeks to induce MetS, and we used a mouse neuronal cell line (HT22) on hydrogels resembling brain tissue stiffness (physiological (~6.5-7.5kPa) or dementia (2.5/2.0kPa)).We found decreased levels of proteins related with brain stiffness in WD-fed mice (α-SMA, cofilin 30% and CTGF 30%) but no alterations on mitochondrial markers (TOM20, VDAC, ATP5). However, we noticed increased levels of glucose metabolism markers (GLUT1 200%, HK 30%), and upregulation of mitochondrial beta-oxidation marker CPT1 (125%) in WD-fed mice brains. In vitro, we confirm that substrate stiffness influences cellular mechanotransduction signals (p-cofilin/cofilin, YAP subcellular localization). Interestingly, mitochondrial or fatty acid-oxidation markers (TOM20, ATP5, CPT1) were not altered, while an overall decay of protein levels associated with metabolism (Complex IV 25%, HADHA 55%, HK 80%) was observed in cells cultured on soft substrate (2.5kPa).

This study suggests that primary alterations in brain lipid metabolism may lead to subsequent changes in brain tissue mechanics in mice with WD-induced MetS. If it is beneficial for the work, the abstract can be supplemented by a picture as follows.

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The mitochondria-targeted antioxidant AntiOxCIN4 improves cardiac oxidative/nitrosative stress in the amyotrophic lateral sclerosis *SOD1*^{G93A} mouse

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Abstract:

Amyotrophic lateral sclerosis (ALS) is a fatal adult-onset neurodegenerative disease, defined by motor neuron loss, muscle atrophy, and paralysis. ALS patients often present severe alterations in cardiovascular function, which may evolve into heart failure and death. ALS pathophysiology remains unknown, but mitochondrial dysfunction and oxidative stress may constitute key players. Hence, improving mitochondrial function and/or antioxidant defenses with novel mitochondria-targeted antioxidants (*e.g.,* AntiOxCIN4) may constitute promising therapies against ALS progression. We hypothesized that the mitochondria-targeted antioxidant AntiOxCIN4 can mitigate cardiac oxidative/nitrosative stress in *SOD1*^{G93A} ALS mice.

Early adult *SOD1*^{G93A} ALS mice were injected subcutaneously with AntiOxCIN4 (0.1 mg/Kg/day), for 2 months. We used heart homogenates and colorimetry-based methods to assess the effect of AntiOxCIN4 in nitrosative stress markers and in the activities of the antioxidant enzymes superoxide dismutase (total superoxide dismutase and superoxide dismutase-2), glutathione peroxidase and reductase.

We observed that AntiOxCIN4 treatment resulted in a slight reduction of 39 % in cardiac nitrites levels and in a significant increase in the activities of total superoxide dismutase, superoxide dismutase-2, glutathione reductase, and peroxidase in the hearts of ALS mice (P=0.05, P=0.03, P=0.008, P=0.0002, respectively).

Our preliminary results suggest that peripherally administered AntiOxCIN4 may enhance cardiac antioxidant defenses and reduce oxidative/nitrosative stress upon ALS. However, further studies are required to uncover if this protection delays ALS-related cardiac damage.

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Unravelling the role of lipid metabolism of Unconventional T cells

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Abstract:

Lifestyle and intestinal inflammation are key risk factors for colorectal cancer. Despite advances in tumoral millieu knowledge, how immune cells sense environmental factors and its impact in malignancy remains largely unknown. Intraepithelial lymphocytes (IELs) comprise a set of specialized T cells that live in intimate contact with the intestinal epithelium, work as the first line of immune defence and preserve epithelial integrity. Interestingly, IELs were recently shown to play a role in systemic metabolism and showed upregulated levels of enzymes involved in cholesterol biosynthesis, when compared with T cells in periphery. Among IELs, there exist unconventional T cells, namely CD4- CD8β- TCRαβ+ CD8αα (CD8αα IELs), also known as natural IELs, which attain an activated and functional phenotype during self-agonist selection within the thymus and subsequently migrate to the intestinal epithelium without requiring peripheral activation. Our laboratory showed maternal retinoids are crucial for innate lymphoid cells and offspring long term immune defence. Recently, we showed that CD8αα IELs require micronutrient vitamin A signaling for thymic development and intestinal maintenance. However, the developmental program of these cells remains unclear, as well as their metabolic profile and its impact on the intestinal immunophysiology remains largely unexplored.

This project aims to understand the role of lipid metabolism in unconventional T cells CD8αα IELs and their function in intestinal mucosa. For that we will: 1) identify and characterize lipid pathways in CD8αα IEL; 2) investigate lipid signalling function in CD8αα IEL; 3) explore intestinal function of lipid-regulated CD8αα IEL.

We will employ state-of-the-art research tools, including genetic, cellular and molecular, including in vitro and in vivo, to identify, quantify and manipulate lipid pathways in CD8αα IEL that may be critical for their function. In addition, by utilizing tissue-specific genetic loss of function mouse models (genetic Cre/LoxP systems) available in the laboratory we will investigate upstream signals that may regulate lipid metabolism in CD8αα IELs.

Thus, by unravelling how lipid metabolism impacts the development and shape of the immune system, namely unconventional T cells, the results obtained in this project will be key for developing new strategies to improve the well-being of people, opening new doors in therapeutic strategies for intestinal malignant diseases.