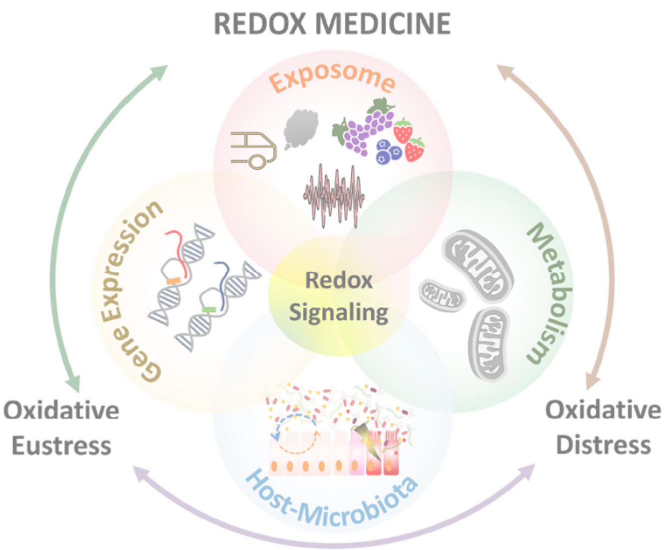
2nd FEBS
REDOX MEDICINE
WORKSHOP

Connecting the exposome with redox regulation in health and disease



PROGRAMME AND ABSTRACT BOOK

October 6 – 9, 2025
Luso, Portugal

The 2nd FEBS Redox Medicine Workshop is a FEBS advanced course organised by Ana Ledo and Bárbara S. Rocha with the support of the following institutions:

The Federation of European Biochemical Societies (https://www.febs.org) in collaboration with The Portuguese Society of Biochemistry (http://www.spb.pt).

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Editors:

Ana Ledo
Bárbara S. Rocha

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Welcome note

Welcome to the 2nd FEBS Redox Medicine Workshop: connecting the exposome with redox regulation in health and disease

Dear participants,

The 2nd FEBS Workshop on Redox Medicine, is dedicated to the theme *connecting the exposome with redox regulation in health and disease*, reflecting the importance of the emerging knowledge on the multifaceted interaction with the external environment, collectively termed the exposome. The workshop will cover the most recent data on the redox regulation of biological functions ranging from fundamental to clinical research as well as therapeutic applications also including sessions on the specific and sensitive tools and techniques to identify and quantify biological oxidants in complex biological settings properly.

Redox reactions are central to life and although the role of Redox Biology in health and disease has many times been reduced to understanding the homeostasis of reactive oxidants, several biochemical pathways include redox reactions and cycling of redox couples which are key in regulation of cell metabolism, cell signaling, and ultimately, health and disease.

In this Redox Medicine Workshop you will have the chance to interact with prominent senior researchers in the field of Redox Biology as well as with young pre- and postdoctoral researchers selected from all over the world. The program has been tailored to promote an informal and relaxed environment in which science, scientific careers and personnel experiences as a scientist in the XXIst century may be discussed and hopefully you will bring new ideas back to your lab and open new avenues on your research career.

We wish you a pleasant and fruitful meeting!

Ana Ledo
Bárbara S. Rocha

Information

Language

The official language of the workshop is English. There will be no simultaneous translation.

Certificate of attendance

The certificate of attendance will be downloadable from your participant account on the Congress website at https://redoxmedicine2025.febsevents.org upon completing the survey at the end of the YSF meeting.

Internet connection

Information on site.

Meals

All meals, excluding coffee breaks, will take place at the Hotel restaurant in the workshop-dedicated area.

Shuttle from Luso to Coimbra-B train station

It will be provided a shuttle from Grande Hotel de Luso to Coimbra-B train station on Friday 10. The bus will leave at 9 a.m. from the hotel entrance.

Programme

Monday, October 6

15:00–17:00	Registration
17:00	Welcome address Fernando Ramos, Faculty of Pharmacy University of Coimbra Giuseppe Valacchi, President of SFRRE Ana Ledo, Chair
17:15	*Chair: Ana Ledo* ***Opening Lecture*** - Helmut **Sies**, Germany *Redox Signaling, Oxidative Eustress and Distress*
18:15	Get together party
20:00	Dinner

Tuesday, October 7

07:00	Breakfast
09:00	**Cardiovascular Pathophysiology** *Chair: Giovanni Mann* Amrita **Ahluwalia**, UK *The non-canonical pathway for NO: key role for xanthine oxidoreductase*
09:30	Giovanni **Mann**, UK *KEAP1/NRF2 mediated redox signaling modulated by the dietary isothiocyanate sulforaphan*
10:00	Selected Oral Communications Session 1 *Chair: Bárbara S. Rocha* Shani **Doron**, Israel *SPEAR-based redox proteomics reveals opposing 2-Cys Prx-mediated redox signals during photosynthetic induction*
10:15	Cátia **Domingues**, Portugal *The Exposome-Redox Interface in Oral Cancer: Mechanistic Insights into Rosmarinic Acid Action*
10:30	Joshua **Ratliffe**, UK *Investigating Oxidation-Induced Formation and Structure of Disulphide-Linked Conjugates by p62-SQSTM1*
10:45	*Coffee break*

11:15	**Nutrigenomics: Diet-driven Modulation of Gene Expression** *Chair: Bárbara Rocha* Carsten **Carlberg**, Poland *Nutrigenomics: Redox Regulation and Epigenomics*
11:45	Agnieszka **Bartoszek**, Poland *The comparison of antioxidant properties and nutrigenomic redox-related activities of vitamin C, C-vitamers, and other common ascorbic acid derivatives*
12:15	Oral Communications from selected abstracts Session 1 *Chair: Ana Faria* Giuseppe Tancredi **Patané**, Italy *Evaluating the Role of Callistemon citrinus anthocyanin-enriched fraction in supporting Leydig Cell function and steroid Biosynthesis*
12:30	Beatriz **Paiva**, Portugal *Dietary Nitrate as a Modulator of Host–Microbiota Redox Interactions Under Antibiotic-Induced Dysbiosis*
12:45	Claire **Fayad**, Finland *Temporal Dynamics of Ultrafine Particle Toxicity and Age-Related Susceptibilities in Human Olfactory Mucosal Cells*
13:00	Lunch
15:00	**Gut feelings** *Chair: Ana Ledo* Ana **Faria**, Portugal *Diet Microbiome Interactions and their Role in Regulation of Intestinal Health* Juan **Sastre**, Spain *Redox signaling in the gastrointestinal tract*

16:00 – 18:30	Rozhica Bogeska, FEBS Press Jan Riemer, FEBS Member in Charge Guided Poster Tour
20:00	Dinner

Wednesday, October 8

07:00	Breakfast
09:00	**Environmental interactions** *Chair: Bárbara Rocha* Tilman **Grune**, Germany *Aging and Environmental Factors: Effects of Nutrition*
09:30	Giuseppe **Valacchi,** Italy *Cutaneous oxinflammatory responses to pollution*
10:00	Oral Communications from selected abstracts Session 3 *Chair: Cátia Domingues* Djurdja **Jerotić**, Serbia *The association of gene polymorphisms of proteins involved in the regulation of redox homeostasis with the development of neurological post-COVID sequelae*
10:15	Stefano **Putaggio**, Italy *Protective effects of Mulberry leaves extract on blood cells*
10:45	Coffee break
11:15	**Redox Cycles and Small Oxidants in Signaling** *Chair: Giovanni Mann* João **Laranjinha**, Portugal *Neuronal nitric oxide: a suitable spatial signal to couple local neural activity in the brain to local blood flow*

11:45	Antonio **Martínez-Ruiz**, Spain *Hypoxia signalling by reactive oxygen species. What can be the relation of celular sodium movements with redox signalling?*
12:15	Selected Oral Communications Session 4 *Chair: Giuseppe Valacchi* Kyana **Van Acker**, Belgium *A novel approach for modelling oxidative stress in the retinal pigment epithelium: characterization of a DAO RPE cell line*
12:30	Emma **Delanote**, Belgium *Exploring the role of a long non-coding RNA in the retinal pigment epithelium and the oxidative stress response, a major disease mechanism in retinal diseases*
12:45	Daniela **Santos**, Portugal *Establishing a Redox Profile for Aging: Biomarker-Based Insights from the ALFA Score Project*
13:00	Lunch
15:00 – 16:00	**Career Development Session: Communication in Science and Outreach** Anthony **Newman** *Senior Publisher with Elsevier* *How to prepare your manuscript and get it accepted in a good journal* Announcement of flash presentation winners
16:00-18:00	Free time
20:00	Dinner

Thursday, October 9

07:00	Breakfast
09:45	**Energy Metabolism** *Chair: Antonio Martinez-Ruiz* Aleksandra **Janković**, Serbia *Integrating Redox-Metabolic Regulation of Adipose Tissue within the Exposome Framework: From Cold Exposure to Obesity and Cancer*
10:15	Carlo **Viscomi**, Italy *Mitochondrial medicine and translational aspects of mitochondrial diseases*
10:45	Coffee break
11:15	**Lipid metabolism and redox signalling** *Chair: Juan Sastre* Maija **Dambrova**, Latvia *Acylcarnitines: Linking Fatty Acid-Derived Energy Metabolites to Diet, Lifestyle, and Redox Balance*
11:45	Irundika **Dias**, UK **Age-related alterations in sulphated oxysterols in human plasma**
12:15	Selected Oral Communications Session 5 *Chair: Bárbara S. Rocha* Camila **Portugal**, Portugal *Vitamin C uptake rewires microglial redox state to modify Alzheimer's disease*

12:30 12:45	Camila **Aburto**, Chile *Metabolic early-warning system: oxidative bursts instantly reroute glucose to safeguard NADPH* Hans **Vellama**, Estonia *Respiration without oxygen - reactions driving CO2 emission in anoxia*
13:00	Lunch
15:00 – 16:00	Flash presentations from selected posters
16:00	**Closing Lecture** *Chair: Ana Ledo* Andreas **Daiber**, Germany *Connecting the exposome and redox signalling in health and disease*
17:00	Closing and Concluding Remarks Group Photo
20:00	Workshop Dinner at Grande Hotel de Luso

Invited speakers

Monday, October 6

Redox Signaling, Oxidative Eustress and Distress

H. Sies[I,II]

[I]*Heinrich Heine Universit, Inst. Biochem. Mol. Biol. I, University Street 1, Düsseldorf, Germany*

[II]*Leibniz Research Institute for Environmental Medicine, Düsseldorf, Germany*

This presentation discusses redox signaling and its role in stress responses to endogenous (metabolic) and exogenous (exposome) challenges. Redox reactions are linked to fundamental life processes, requiring constant monitoring of the flow equilibria in the open metabolic system. Consequently, a physiological level of reactive signaling molecules addresses specific targets to initiate adaptive responses, "eustress", whereas high (supraphysiological) or low (subphysiological) exposure leads to disrupted redox signaling and unspecific molecular damage, "distress". Current knowledge on major concepts in redox organization, molecular players (focus on protein thiols and hydrogen peroxide) and of some life processes is presented.

Tuesday, October 7

The noncanonical pathway for NO: key role for xanthine oxidoreductase

A. Ahluwalia

Barts & The London Faculty of Medicine & Dentistry, Queen Mary University of *London, Charterhouse Square, London, United Kingdom*

In contrast to NO, which has welle stablished, and important roles in the regulation of blood flow and blood pressure its oxidative metabolites nitrite (NO2) and nitrate (NO3) were considered to be relatively inactive. This view has been radically revised over the past two decades. Much evidence now demonstrates that nitrite serves as a storage form of NO, releasing NO under acidic and/or hypoxic conditions but also under physiological conditions: a phenomenon that is catalyzed by a number of distinct mammalian nitrite reductases. Importantly, preclinical studies demonstrate that reduction of nitrite to NO results in vasodilatation of blood vessels and lowering of blood pressure and cytoprotective effects that protect the heart, this latter issue

having been translated more recently to the clinical setting. In addition, research has demonstrated that the other main metabolite of the oxidation of NO i.e. nitrate can also be sequentially reduced through processing in vivo to nitrite and then nitrite to NO to exert a range of beneficial effects, most notably also lowering of blood pressure; a phenomenon that has also been confirmed recently to be an effective method for blood pressure lowering in patients with hypertension. In this lecture I will provide a detailed description of the pathways involved in the bioactivation of both nitrate and nitrite in vivo, their functional effects in preclinical models, their mechanisms of action and discussion of translational exploration of this pathway in cardiovascular disease.

KEAP1/NRF2 mediated redox signaling modulated by the dietary isothiocyanate sulforaphane

S. J. Chapple[I], **G. Mann[I]**

[I]King's British Heart Foundation Centre of Research Excellence, School of Cardiovascular and Metabolic Medicine & Sciences, King's College London, London, United Kingdom

The dietary isothiocyanate sulforaphane (SFN) has been the focus of randomized clinical trials and, although not all have proven successful, emerging evidence suggests SFN may afford health benefits in the treatment of diabetic and cardiometabolic diseases across the life course (Patel et al., Free Radic Biol Med. 2018;122:150160). SFN is a potent inducer of the redox sensitive KEAP1/NRF2 pathway and its downstream cellular antioxidant defenses (Fahey et al., Proc. Natl. Acad. Sci. USA 1997; 94;10367–10372). Protective actions of SFN may also be mediated via modulation of proinflammatory (NFκB) and metabolic (PPARγ) signaling pathways. We previously reported that NRF2 activation is impaired in fetal umbilical vein endothelial cells cultured from gestational diabetic pregnancies (Cheng et al., Diabetes 2013;62;4088–4097). Our studies in a murine model of obese pregnancy established that maternal dietary administration of SFN induces NRF2 activation and attenuates redox imbalance in utero and improves early life cardiovascular function in offspring (Psefteli et al., Redox Biology 2023;67:102883). Notably, protection afforded by SFN against redox distress in utero was only observed in wildtype but not NRF2 deficient mice. With respect to age related disease, SFN protects against ischemia/reperfusion induced increases in blood brain barrier permeability and behavioural deficits in rodent models of ischemic stroke (Alfieri et al., Free Radic Biol Med. 2013;65:10121022, Srivastava et al., J Physiol. 2013;59:352538). Our studies in brain microvascular endothelial cells adapted long term (5 days) to hyperoxic (18 kPa) or normoxic (5 kPa) O2 levels established that NRF2 mediated induction of HO1 and GCLM by SFN (2.5µM) was significantly attenuated in cells adapted to 5 kPa O2 despite NRF2 nuclear accumulation (Warpsinski et al., Redox Biology 2020;37:101708). As redox and other intracellular signaling pathways are critically affected by O2, high throughput screening of therapeutics targeting the KEAP1/NRF2 pathway in cell models in vitro should be conducted under well defined O2 levels to improve clinical translation for adverse pregnancy and age related diseases such as stroke, coronary and renal disease. Supported by Henry Smith Charity, Heart Research U.K. (RG2672), British Heart Foundation (PG/24/1159, FS/16/67/32548, FS/13/66/30445, PG/17/38/33024, FS/20/8/34984) and support in kind provided by Baker, BMG Labtech, Lucid Scientific and Nanion Technologies. We gratefully acknowledge our collaborators and coauthors in the cited publications.

Nutrigenomics: Redox Regulation and Epigenomics

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C. Carlberg[I,II]

[I]ERA Chair of Polish Academy of Sciences, Olsztyn, Poland, [II]University of Eastern Finland, Kuopio, Finland

Nutrigenomics explores how dietary components modulate gene expression through interactions with the genome and epigenome. Among the most biologically active dietary molecules is vitamin D , which, through its active metabolite 1α,25-dihydroxyvitamin D , binds the vitamin D receptor (VDR), a nuclear, ligand inducible transcription factor expressed in most human tissues. VDR modulates chromatin accessibility at thousands of genomic loci, leading to tissue-specific up- or downregulation of gene expression. A proof of principle in vivo intervention demonstrated that high dose vitamin D supplementation rapidly affects gene expression in peripheral blood mononuclear cells (PBMCs), including key redox regulatory genes such as PRDX1, TXNRD1, SOD2, and SELENOS. These genes are involved in antioxidant defense and the selenium micronutrient network, underscoring the immediate impact of nutritional signals on redox homeostasis. Beyond vitamin D, many nutrient driven gene environment interactions are redox sensitive and epigenetically mediated. The cellular redox state, reflected by factors such as the NAD /NADH ratio or vitamin C levels, influences histone acetylation and DNA methylation patterns, shaping both transient and long lasting epigenomic changes. Such modifications not only affect cellular function but may persist over time, contributing to health trajectories or disease risk, and even influencing transgenerational inheritance. Dietary signals are continuously integrated by the epigenome throughout life, from conception to aging. While these mechanisms maintain homeostasis under healthy conditions, maladaptive responses to persistent dietary imbalances, such as those observed in obesity, can drive chronic diseases including type 2 diabetes and cancer. In my lecture, I will discuss how nutrigenomics bridges nutrition, redox biology, and epigenetics. Vitamin D will serve as a paradigmatic example to illustrate how nutrition derived signals dynamically regulate immune function and oxidative stress responses at the systems level, providing insights into precision nutrition strategies for health maintenance and disease prevention.

The comparison of antioxidant properties and nutrigenomic redox related activities of vitamin C, C-vitamers, and other common ascorbic acid derivatives

A. Bartoszek[I], P. Jakubek-Olszewska[II], M. Kuczyńska[III], K. Suliborska[III], K. Parchem[III], I. KossMikołajczyk[III], W. Chrzanowski[III], J. Namieśnik[III]

[I]Gdansk University of Technology, 11/12 Narutowicza St, Gdansk, Poland, [II]Gdansk University of Technology/Nencki Institute of Experimental Biology, Gdansk/Warsaw, Poland, [III]Gdansk University of Technology, Gdansk, Poland

Under the name ‘vitamin C’ hides a group of compounds with antiscorbutic activity of L-ascorbic acid. Apart of their natural presence in plant-derived foods, some derivatives of L-ascorbic acid are widely implemented in the food industry as antioxidants, including the D-isomers, which lack vitamin C activity. Our research concentrated on the relationship between chemical structure, electrochemical properties and redox-related bioactivities for most common of these derivatives. Within physiological range of concentrations, there was no simple relationship between their redox properties and biological activity. Clear distinctions in antioxidant activity were observed mostly at high concentrations, which were strongly correlated with electrochemical and kinetic parameters describing redox-related properties of the studied compounds. Despite obvious similarities in chemical structures and antioxidant activity, we showed that ascorbic acid analogues may exhibit different nutrigenomic effects. This notion about diverse redox-related biological behaviour of structurally related reducing compounds is true also for other dietary antioxidants we investigated.

Diet Microbiome Interactions and their Role in Regulation of Intestinal Health

A. Faria[I,II]

[I]Nova Medical School|Faculdade de Ciências Médicas Universidade Nova de Lisboa, Lisboa, Portugal, [II]CHRC – Comprehensive Health Research Center, Lisboa, Portugal

Diet strongly shapes the gut microbiome, whose composition and metabolites are key regulators of intestinal barrier integrity, immune balance, and systemic metabolism. Dysbiosis disrupts these processes, promoting bacterial translocation and proinflammatory signalling linked to metabolic, neurological, and autoimmune disorders. Beyond microbial composition, metabolites such as short-chain fatty acids, indole derivatives, or kynurenine intermediates mediate critical host–microbiota interactions. Emerging evidence highlights that this metabolic dialogue extends to mitochondria. Mitochondrial dysfunction often cooccurs with dysbiosis and is recognized as a hallmark of noncommunicable diseases. Yet, the bidirectional communication between microbiota and mitochondria remains underexplored. Nutritional and microbial interventions—including targeted nutrients, probiotics, prebiotics, symbiotics, and fecal microbiota transplantation—hold promise for restoring eubiosis and mitochondrial function. Together, diet–microbiome–mitochondria interactions emerge as a powerful framework for maintaining intestinal health and preventing NCDs.

Redox signaling in the gastrointestinal tract

J. Sastre[I]

[I]Department of Physiology, Faculty of Pharmacy, University of Valencia, Burjassot (Valencia), Spain

Redox signaling regulates physiological self-renewal, proliferation, migration and differentiation in gastrointestinal epithelium by modulating Wnt/βcatenin and Notch signaling pathways mainly through NADPH oxidases (NOXs). Importantly, commensal bacteria contribute to intestine epithelial homeostasis through NOX1and dual oxidase 2-derived reactive oxygen species (ROS). The loss of redox homeostasis is involved in the pathogenesis and development of a wide diversity of gastrointestinal disorders, such as Barrett's esophagus, peptic ulcer, inflammatory bowel disease, colorectal cancer, acute pancreatitis, and pancreatic cancer. The overproduction of superoxide anion together with inactivation of superoxide dismutase contribute to the pathogenesis of Barrett's esophagus and its transformation to adenocarcinoma. In Helicobacter pylori-induced peptic ulcer, oxidative stress derived from the leukocyte infiltrate and NOX1 aggravates mucosal damage, particularly in HspB+ strains that downregulate Nrf2. Progression of inflammatory bowel disease relies on the balance between proinflammatory redox-sensitive pathways, such as NLRP3 inflammasome and NF-kappaB, and the adaptive upregulation of Mn superoxide dismutase and glutathione peroxidase 2. In colorectal cancer, redox signaling exhibits two Janus faces: On the one hand, NOX1 upregulation and derived hydrogen peroxide enhance Wnt/βcatenin and Notch proliferating pathways; on the other hand, ROS may disrupt tumor progression through different proapoptotic mechanisms. Glutathione depletion, disulfide stress, mitochondrial ROS, and nitrosative stress contribute to the pathogenesis of acute pancreatitis. Mitochondrial ROS also cooperates with mutant KRAS to trigger acinar-to-ductal metaplasia and progression to pancreatic adenocarcinoma. Therefore, redox signaling plays a fundamental role in the physiology and pathophysiology of gastrointestinal tract.

Wednesday, October 8

Aging and Environmental Factors: Effects of Nutrition

T. Grune[I]

[I]German Institute of Nutrition, Potsdam-Rehbrücke, Nuthetal, Germany

Aging is inevitable associated with metabolic alterations. Redox balance disturbances and severe oxidation are often leading to irreversible protein modification. If not degraded in time, such protein material accumulates. Such accumulated protein aggregates – also referred to as lipofuscin – have a number of metabolism-modulating effects, often enhancing the aging process itself. It is well established that postmitotic cells are especially prone to accumulate lipofuscin, among those neurons, skeletal muscle and cardiomyocytes and also ß-cells. Although this is known for some time, the metabolic and functional consequences of lipofuscin accumulation are still under investigation. Especially, the influence of nutrition and the metabolic state on this aspect of the aging process is hitherto not well understood. Our studies focused on the investigation of ß-cells and cardiomyocytes and the influence of nutrition on their metabolism and function in various age stages with special emphasis on the role of lipofuscin accumulation.

Cutaneous oxinflammatory responses to pollution

G. Valacchi[I,II,III]

[I]Department of Environmental and Prevention Sciences, University of Ferrara, Ferrara, Italy, [II]Department of Animal Sciences, Plants for Human Health Institute, NC State University, Kannapolis, United States of America, [III]Department of Food and Nutrition, Kyung Hee University, Seoul, South Korea

As the most apparent organ subjected to external environmental stressors, the impact of pollution on the skin has been extensively researched over the past few decades. While ultraviolet (UV) radiation is recognized as the most harmful stressor that our skin encounters on a daily basis, various elements of tropospheric pollution have also been demonstrated to influence skin health and its functions. Notably, agents such as ozone, particulate matter, cigarette smoke, and micro/nano plastics have been identified as factors that can alter skin homeostasis. Furthermore, the interplay among various pollutants has garnered significant attention in current research.

Multipollutant exposure has been shown to induce skin barrier dysfunction leading to oxidative damage and the activation of an inflammatory cascade (canonical and not canonical inflammasome activation) in human skin. Topical application and dietary interventions have shown in some cases a clear protective effect against oxinflammatory pollution skin damage. Having a better understanding of pollutome effects on skin barrier properties and oxinflammatory responses could help to design innovative protective solutions and develop specific topical and/or dietary regimes to protect the skin.

Neuronal nitric oxide: a suitable spatial signal to couple local neural activity in the brain to local blood flow

J. Laranjinha[I]

[I]*Faculty of Pharmacy and Center for Neurosciences and Cell Biology, Coimbra, Portugal*

Communication between active neurons and vascular cells is essential for maintaining brain structure and function. This is largely because the brain is critically dependent on the rapid and transient delivery of bioenergetic substrates (oxygen and glucose) by the local vasculature to neighboring cells, with spatial and time precision, according to energy demands imposed by neural activation. Failure in this process, known as neurovascular coupling, either due to aging, disease (Alzheimer´s disease) or acute hypoxic events, can compromise brain integrity and impair cognitive performance. Although no single mechanism is likely responsible for neurovascular coupling, we and others have previously supported that, mechanistically, the communication between neurons and vascular cells is mediated, at least in part, by volume signaling involving nitric oxide (NO) of neuronal origin. Unlike other diffusible informational molecules, NO is small and hydrophobic enough to readily permeate biological membranes. It is not stored but diffuses in three dimensions away from its site of synthesis, across cell membranes down a concentration gradient through cytoplasm, membranes and extracellular fluid. In doing so, NO coordinates the activity of a group of cells in a volume of brain tissue governed by its half-life and diffusion properties. Notably, in addition to endogenous NO synthesis, a novel mechanism has been proposed that links environmental factors (diet) to NO biology via the nitrate:nitrite:NO pathway. The concept of NO as diffusible intercellular messenger, linking space and time in the brain to support neurovascular coupling and cognition, will be explored in the context of animal models of cognitive impairment.

Hypoxia signalling by reactive oxygen species. What can be the relation of celular sodium movements with redox signalling?

A. Martínez-Ruiz[1]

[1]Instituto de Investigación Sanitaria Princesa (IIS Princesa), Madrid, Spain

Oxygen is fundamental for eukaryotic life, and at the same time is the source for reactive oxygen species, which can act as signalling molecules in what we call redox signalling. Cells and organisms have developed specialized mechanisms to sense and trigger different adaptive responses to the reduced availability of oxygen (hypoxia). In our research group we have been interested in studying what are the redox signalling pathways that mediate these hypoxic responses in mammalian cells in different pathophysiological scenarios. Once upon a time in the lab we established that different cell types produce a superoxide burst from mitochondrial origin in response to acute hypoxia that could be used as a redox signal. Searching for the mitochondrial mechanisms producing this superoxide signal, we uncovered an unexpected role of mitochondrial Na^+ import as a key signal transducer in this process, mediated by the mitochondrial $Na^+/Ca^{2+}/Li^+$ exchanger NCLX and exerted by a biophysical mechanism of Na+ binding to the inner mitochondrial membrane that regulates electron transport chain behaviour.

We have explored the implications of this redox signalling mechanism triggered by mitochondrial Na^+ import in different physiological responses to hypoxia, such as acute hypoxic pulmonary vasoconstriction and the activation of the hypoxia-inducible factors (HIF) pathway regulating hypoxic gene expression signatures. We have also found that NCLX activity is also important in nonhypoxic processes such as activation of the NLRP3 inflammasome, probably by a redox signalling mechanism. Using novel pharmacological candidates developed in our Institute, we have searched for possible therapeutic benefits of inhibiting this NCLX-dependent redox pathway in situations of oxidative stress linked to ischemia and reperfusion such as in cellular and animal models of ischemic stroke.

How to Get your Research Published in a Good Journal: Tips and tricks

A. Newman[I]
[I]*Elsevier, Amsterdam, Netherlands*

Background: Knowing the best way of structuring your manuscript when writing it, and the most appropriate journal to send it to, really helps in getting your paper accepted. Also understanding how editors and publishers think and what they expect from you, and knowing how the peer review process works, is an invaluable insight into the publishing process.
Results: After attending this workshop, participants will have a clear idea of the steps needed to be taken before starting to write a paper including selecting the most appropriate journal to submit to – including open access or not. Authors will also be able to plan writing manuscripts using the logical step sequence – not the sequence in which the paper will be read. The correct ethical use of Open AI is also important as it is a useful tool but easily misused. Authors are also made aware of what aspects of their papers Editors, Reviewers, and Publishers look at critically, and to ensure that in taking care of these areas, their papers are much more likely to be accepted. Dealing with referees' comments and the art of polite rebuttal are also covered such that these can be used to improve the submitted paper suitably. Sensitive areas such as publishing ethics, plagiarism, duplicate publishing, etc. are also clearly explained such that participants have a better understanding of what their responsibilities are, what is allowed, and what is not permitted.
Conclusions: These insights into the publishing process will enable each participant to be more confident as an author in the world of science publishing, and so should help them get their papers published more easily.
Keywords: Scientific Publishing, Paper authorship, ethics, journal.

Thursday, October 9

Integrating Redox-Metabolic Regulation of Adipose Tissue within the Exposome Framework: From Cold Exposure to Obesity and Cancer

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T. Zakić[I], A. Korać[II], B. Korać[I], **A. Janković[I]**

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The exposome framework, encompassing external (like diet, temperature) and internal (such as metabolic stress) environmental exposures, highlights adipose organ as a key interface for energy and redox processes influencing metabolism. Exposure to low environmental temperatures triggers brown adipose tissue (BAT) to activate non-shivering thermogenesis. This process harnesses energy from food and mobilizes lipids from white adipose tissue (WAT), all coordinated through intricate redox processes to effectively sustain heat production. Mitochondrial reactive oxygen species (ROS) are crucial in mediating thermogenic activation and uncoupling protein 1 in BAT. ROS and reactive nitrogen species also promote WAT browning into beige adipocytes, offering anti-obesity strategies. However, in obesogenic states, WAT shows redox imbalance linked to oxidative damage, mitochondrial dysfunction, disrupted insulin signaling, and impaired adipokine production, increasing risks of insulin resistance and metabolic disorders. In obesity-related cancers like breast cancer, adipose organ undergoes changes, with tumors exploiting cancer-associated adipose tissue (CAAT) via redox and metabolic rewiring to access nutrients supporting growth. The recent studies of breast cancer reveal Nrf2's role in mediating redox coupling between tumors and CAAT. Loss of Nrf2 disrupts this communication, redox balance, and alters tumor mitochondrial dynamics, emphasizing bidirectional interactions governed by Nrf2. These findings show the exposome as a valuable perspective on redox-metabolic regulation in adipose organ in health and disease, from cold exposure to obesity and cancer. Incorporating the exposome into adipose and cancer research offers a path to precision redox medicine for treating metabolic diseases, including cancer.

Acknowledgement: This work was supported by the Science Fund of the Republic of Serbia, project #7750238 “Exploring new avenues in breast cancer research: Redox and metabolic reprogramming of cancer and associated adipose tissue” REFRAME *and the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, (grant #45103137/202503/200178 and #45103136/202503/200007).*

Mitochondrial medicine and translational aspects of mitochondrial diseases

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Mitochondrial myopathies are genetic disorders caused by mutations in mitochondrial or nuclear DNA, leading to defective oxidative phosphorylation and severe muscle dysfunction. While no cure currently exists, promising therapeutic strategies are emerging, including pharmacological and gene therapy approaches. Our previous work demonstrated that rapamycin, an mTORC1 inhibitor, improved motor function and reduced myopathy in a Cox15 musclespecific knockout (Cox15sm) mouse model by activating TFEB-mediated autophagy and lysosomal biogenesis. Given rapamycin's broad systemic effects, we explored alternative TFEB activators, such as Urolithin A (UA), a natural polyphenol known to stimulate mitophagy. Cox15sm mice expressing the mitophagy reporter MitoQC were treated with UA for nine weeks. By the end of the study, treated mice exhibited significant motor improvement, enhanced mitophagy in skeletal muscle, and improved mitochondrial ultrastructure, although cytochrome c oxidase (COX) activity remained unchanged. In parallel, we investigated AAV-based gene therapy using a novel liver-detargeted, myotropic AAV vector to restore Cox15 expression in Cox15sm mice. Following systemic AAVCox15 administration, treated mice showed a marked increase in motor performance—up to tenfold improvement—beginning as early as two to four weeks postinjection.
Muscle histology confirmed reduced myopathy and partial restoration of COX activity. EM analysis showed rescued mitochondrial ultrastructure. Importantly, this new AAV vector demonstrated superior efficacy compared to AAV9 at equivalent doses. Together, these studies highlight two complementary therapeutic strategies: UA as a pharmacological approach to enhance mitophagy and an optimized AAV gene therapy for direct genetic correction. These findings pave the way for further exploration of combinatorial or standalone treatments for mitochondrial myopathies

Acylcarnitines: Linking Fatty Acid-Derived Energy Metabolites to Diet, Lifestyle, and Redox Balance

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Acylcarnitines, the esters of carnitine and fatty acids, are key intermediates of fatty acid metabolism. While the fundamental role of carnitine in mitochondrial long-chain fatty acid transport is well established, the physiological functions of specific acylcarnitines and the significance of their chain-length diversity remain only partly understood. L-carnitine, discovered more than a century ago by Latvian biochemist R. Krimberg, is both an essential biofactor for mitochondrial β-oxidation and a widely used nutritional supplement, valued for its safety and potential effects on energy metabolism. However, efficient stimulation of fatty acid oxidation requires high intracellular concentrations, whereas carnitine bioavailability under normal physiological conditions is relatively limited. Supplementation with high doses of L-carnitine alters systemic metabolite levels, particularly trimethylamine-N-oxide and acylcarnitines. Advances in mass spectrometry now allow comprehensive profiling of carnitine and acylcarnitines, opening new perspectives on their roles in health and disease. Acylcarnitine analysis is already applied in clinical practice, most notably in newborn screening for inborn errors of fatty acid oxidation. Accumulation of specific long-chain or very-long-chain acylcarnitines serves as a biomarker of defective βoxidation enzymes, including mitochondrial trifunctional protein. Physiologically, plasma acylcarnitine concentrations fluctuate with fasting–feeding cycles and the availability of fatty acids and glucose. Elevated long-chain acylcarnitine levels have been shown to impair insulin signaling and mitochondrial function, promoting excessive reactive oxygen species production—effects that are reversible when concentrations decline. Nutritional status and lifestyle factors, including physical activity, also modulate acylcarnitine profiles, emphasizing their dynamic link to metabolism and redox regulation. Growing evidence highlights the importance of defining physiological versus pathological thresholds for acylcarnitines. A deeper understanding of their diverse roles in cellular processes may provide novel biomarkers and therapeutic opportunities, bridging the exposome, redox biology, and metabolic health.

Age-related alterations in sulphated oxysterols in human plasma
IST112

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Background: Sulphated oxysterols are increasingly recognised for their involvement in age-related pathologies, including atherosclerosis and neurodegenerative diseases. However, their metabolic profiles in ageing populations remain poorly characterised due to analytical challenges and unavailability of standards. This study aimed to quantitatively analyse sulphated oxysterols in human plasma and explore their association with cognitive impairment.
Methods: Plasma samples were collected from 153 individuals aged 60 to 85. Lipids were extracted using solid-phase extraction. Samples were then analysed using ultra-high-performance liquid chromatography coupled with SCIEX 5500 QTRAP Mass Spectrometer. Quantification was performed using deuterated internal standards and calibration curves. Statistical analyses, including multivariate regression and principal component analysis, were conducted to assess age-related trends and correlations with clinical biomarkers.
Results and Discussion: Cholesterol sulfate, 25-hydroxycholesterol sulfate, 27-hydroxycholesterol sulfate, and 7-keto cholesterol sulfate was reliably detected and quantified across all samples. Concentrations of 27-hydroxycholesterol sulfate increased significantly with age ($p < 0.05$). 24S-hydroxycholesterol sulfate, was notably higher in older individuals with mild cognitive impairment. This study demonstrates robust quantification of sulphated oxysterols in ageing human plasma using targeted mass spectrometry method. The age-associated increases observed suggest altered cholesterol metabolism and sulfation pathways with ageing, potentially contributing to the development of inflammatory and neurodegenerative conditions. These findings provide new insights into the metabolic shifts in cholesterol oxidation and conjugation in ageing, warranting further longitudinal and mechanistic studies.

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Connecting the exposome and redox signalling in health and disease

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Large epidemiological studies at the global scale, such as the Global Burden of Disease project, have indicated that in 2020 almost two-thirds of the annual global deaths were caused by chronic noncommunicable diseases (NCDs), e.g. atherosclerotic or diabetic sequelae. For 2030, 77% of all deaths are expected to be caused by NCDs. Although this is mainly due to the aging of people in Western societies, other reasons are the increasing impact of soil, water, air and noise pollution, together with other environmental risk factors, such as climate change, unhealthy city design including the lack of green space, unhealthy lifestyle habits and mental stress. The exposome concept was established in 2005 as a new strategy to study the impact of the environment on health. The exposome describes the harmful biochemical and metabolic changes that occur in our body by the totality of different environmental exposures at a lifelong time scale that ultimately lead to adverse health effects and premature deaths. Despite the fact that oxidative stress and adverse redox signalling are hallmarks of the central patho-mechanisms initiated by all environmental risk factors, there is so far a paucity of implementation of redox biomarkers and profound mechanistic studies of oxidative stress-related pathways. Accordingly, the exposome concept will be described with focus on the physical and chemical exposures and on the application of redox biochemical approaches. This will be done by presenting selected exposomic studies in combination with potential redox targets and highlighting their relevance for future health research.

Oral Communications

OC-01

SPEAR-based redox proteomics reveals opposing 2-Cys Prx-mediated redox signals during photosynthetic induction

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Reactive oxygen species (ROS), once considered solely damaging byproducts of metabolism, are now recognized as key signaling molecules that regulate a wide array of cellular processes in both health and disease. Central to this signaling are thiol-based redox modifications of cysteine residues, which can act as reversible molecular switches. Mapping these modifications is essential for understanding redox-regulated networks. We developed SPEAR (Simultaneous Protein Expression and Redox analysis), a novel redox proteomics method enabling site-specific identification and quantification of cysteine oxidation without requiring peptide enrichment. SPEAR employs differential isotopic labeling of reduced and oxidized cysteines with light and heavy Nethylmaleimide (NEM), allowing for parallel analysis of both redox state and protein abundance. We applied SPEAR to Arabidopsis thaliana during the photosynthetic induction phase—the metabolic transition from dark to light—to study the role of 2-Cys peroxiredoxins (2-Cys Prx) in regulation of photosynthesis rates. In wildtype plants, 116 cysteine-containing peptides became reduced and 59 oxidized after 50 seconds of low light, revealing two opposing redox responses triggered by the dark-to-light shift. Photosynthesis-related proteins tended to reduce upon illumination, whereas other metabolic pathways displayed the opposite trend. Interestingly, 2-Cys Prx-deficient mutants (2cpab) showed markedly fewer redox changes, despite similar protein expression profiles, suggesting that 2-Cys Prx orchestrates these dynamic redox shifts. Our findings imply a previously unrecognized layer of regulation that provides spatial specificity within the chloroplast, potentially via the formation of membraneless condensates, offering new insight into the complexity of photosynthetic induction during light transitions. These principles likely extend beyond plants to broader eukaryotic redox systems.

OC-02

The Exposome-Redox Interface in Oral Cancer: Mechanistic Insights into Rosmarinic Acid Action

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Oral squamous cell carcinoma (OSCC) incidence is projected to increase by nearly 40% by 2040, underscoring the urgent need for preventive and therapeutic strategies. Rosmarinic acid (RA), a natural polyphenol with reported anticancer properties, remains poorly explored in OSCC. Here, we investigated RA in both 2D monolayers and 3D spheroid models of OSCC to delineate its mechanistic actions at the exposome–redox interface. RA selectively reduced cell mass and metabolic activity in a dose, time, and cell-type-dependent manner, with the strongest effects in highly invasive OSCC cells, while sparing normal oral mucosa at therapeutic concentrations. Mechanistically, RA decreased mitochondrial membrane potential and altered redox homeostasis, as supported by metabolomic profiling that revealed glutathione depletion alongside the release of acetate and fumarate. RA further induced autophagy, as indicated by BNIP3 and BCNL1 upregulation and BIRC5 downregulation, and modulated epithelial–mesenchymal transition by increasing CADM1 while suppressing VIM, CADM2, SNAIL1, and SOX9 expression. Extracellular matrix remodelling was evidenced by downregulation of MMP2 and MMP9. Molecular docking suggested a high-affinity interaction with P-glycoprotein (–6.4 kcal/mol), while surface charge measurements in HSC3 cells indicated partial reversal of polarity (-22.6 ± 0.3 mV vs. -26.3 ± 0.3 mV, $p < 0.0001$), consistent with impaired invasive capacity. In 3D spheroids, RA reduced both growth and metabolic activity, further supporting its potential as an anticancer agent. Importantly, its modest binding to salivary proteins highlights the feasibility of oro-mucosal delivery. Collectively, these findings position RA as a promising agent targeting redox pathways and cell plasticity in OSCC, warranting further investigation as

monotherapy, in combination regimens, or within nanosystem-based delivery platforms.

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OC-03

Investigating Oxidation-Induced Formation and Structure of Disulphide-Linked Conjugates by p62-SQSTM1

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The selective autophagy receptor p62/SQSTM1 recruits cellular waste for degradation by forming a flexible filamentous helical scaffold, allowing it to bind to both ubiquitinated cargoes and the autophagy core machinery protein LC3. p62 self-oligomerisation is mediated by the PB1 domain, however recent evidence suggests that in oxidative stress conditions, p62 can also form disulphide-linked conjugates (DLCs). This allows p62 to increase autophagic recruitment in response to oxidative stress conditions, including recruitment of dysfunctional mitochondria producing excess reactive oxygen species, thereby restoring redox homeostasis. However, whilst the structure of the PB1 domain-mediated p62 oligomer has already been characterised, the structure of p62 DLCs, and whether they differ from PB1 oligomers, remains uncertain. To investigate the formation of p62 DLCs in vitro, recombinant p62 proteins, consisting of Maltose Binding Protein (MBP) joined to different lengths of p62, have been expressed and purified. Treatment of recombinant protein with the oxidising agent hydrogen peroxide results in DLC formation, as seen on both SDSPAGE gels and

analytical size exclusion chromatography. DLC formation is independent of PB1 oligomerisation, as mutations in the PB1 domain that perturb oligomer formation do not affect the response to hydrogen peroxide treatment. These DLCs can be observed as filamentous structures by negative stain electron microscopy, and more detailed imaging by cryo-EM is currently in progress. By characterising how p62 responds to oxidation and forms DLCs, the mechanism by which selective autophagy is upregulated in response to oxidative stress can be better understood.

OC-04

Evaluating the Role of Callistemon citrinus anthocyanin-enriched fraction in supporting Leydig Cell function and steroid Biosynthesis

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The shift from a linear to a circular economy models aims to find sustainable bioactive compounds with potential clinical uses. Due to their antioxidant properties, polyphenols and flavonoids are considered promising for treating conditions associated with oxidative stress, such as male infertility. This research investigates the impact of an anthocyanin-rich fraction derived from Callistemon citrinus (Lemon bottlebrush) flowers, along with cyanidin 3Oglucoside (cya3O), on the viability, metabolic activity, and steroid hormone production of Leydig cells. A green extraction process was applied to Callistemon citrinus, and its composition was analyzed through RPHPLCDADESIMS/MS, showing a dominant anthocyanin content. TM3 Leydig cells were treated with different concentrations of the anthocyanin-rich fraction (0.01, 0.1, 1 µg/ml) and cya3O (0.5, 5, 50 µM) for 24 hours. Preliminary assessments included cytotoxicity, mitochondrial membrane potential, and reactive oxygen species (ROS) generation. Findings indicated that the extract influenced Leydig cell metabolic activity, increased the production of ROS and caused a reduction in androstenedione levels at higher concentrations, whereas the pure compound did not produce this effect. To better understand these outcomes, the expression of genes involved in steroidogenesis—like STAR, CYP17A1, and NR0B2—was analyzed. Ongoing research involves exometabolome profiling via 1HNMR to comprehensively understand the role of anthocyanin-enriched fractions and cya3O in modifying Leydig cell viability, metabolism, and steroid hormone production.

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OC-05

Dietary Nitrate as a Modulator of Host–Microbiota Redox Interactions Under Antibiotic-Induced Dysbiosis

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The nitrate–nitrite–nitric oxide (NO) pathway comprises sequential reductions in which dietary nitrate is converted to nitrite by oral commensals and subsequently to NO and other bioactive nitrogen oxides in the stomach. Beyond supporting NO bioavailability, this pathway regulates systemic vascular tone, cellular metabolism, and mitochondria efficiency. Given the obligatory role of the microbiota in nitrate-driven redox signaling, the aim of this study was twofold: i) investigate the effect of antibiotic-induced dysbiosis on colonic mitochondrial function, generation of S- and N-nitroso species and cecal nitrate reductase activity and ii) determine the impact of inorganic nitrate intake on these parameters under both eubiosis and dysbiosis. Wistar male rats were randomly distributed in four experimental groups, and the drinking water was supplemented for 7 days as follows: 1) regular drinking water; 2) imipenem (1.25µg/mL); 3) imipenem (1.25µg/mL) + sodium nitrate (10mM); 4) sodium nitrate (10mM). Although there was no significant difference in the concentration of nitrite in the colon mucosa, increased nitrate intake led to a marked rise in S- and N-nitroso species ($p= 0.0352$). Co-treatment with imipenem reversed this effect, with a threefold decrease observed, highlighting the critical role of the gut and oral microbiota in the nitrate–nitrite–nitric oxide signaling pathway. The oxygen consumption rate in colon biopsies collected from each animal was assessed using the oxygraphy Ok2 system (Oroboros) coupled to a substrate-uncoupler-inhibitor-tritration protocol to assess both the N- and S- pathways of the electron transport system. A significant decrease in the maximal activity of cytochrome C oxidase was observed ($p= 0.0125$) under dysbiosis, which was reverted when nitrate was co-administered with the imipenem ($p= 0.0219$). We also observed a significant increase in the ratio between complex II and complex IV activities in the colon biopsies from animals treated with imipenem, suggesting an increased flow in the Krebs cycle and/or a decrease in the flow in the electron transport system. This increase was reversed by nitrate supplementation (0.22 ± 0.12 vs 0.14 ± 0.08). These findings demonstrate that dietary nitrate preserves mitochondrial function and promotes the formation of nitroso species in the gut and this protective capacity depends on an intact

microbiota, thereby reinforcing the pivotal role of the nitrate–nitrite–nitric oxide pathway in maintaining intestinal redox balance during dysbiosis.

OC-06

Temporal Dynamics of Ultrafine Particle Toxicity and Age-Related Susceptibilities in Human Olfactory Mucosal Cells

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Over 99% of the global population is exposed to air pollution exceeding WHO guidelines. Older adults are especially vulnerable, with those aged ≥60 expected to double to 2 billion by 2050. This demographic shift intensifies concern over ultrafine particles (UFPs; < 0.1 µm), a major component of air pollution from diverse sources. Their small size and reactive properties allow them to bypass biological barriers, inducing systemic toxicity especially oxidative stress and neuroinflammation linked to age-related neurodegenerative diseases. However, real-time monitoring and mechanistic understanding of UFP toxicity, particularly in aging populations, remain limited. The olfactory cleft, at the roof of the nasal cavity and directly connected to the brain, serves as a primary entry route for inhaled UFPs and a novel model for neurotoxicity studies. This study investigates source- and time-dependent effects of UFPs on primary human olfactory mucosal (hOM) cells from elderly donors (70–85 years, n=3). UFPs were collected from Nanjing, China (polluted urban air) and Kuopio, Finland (cleaner Nordic air). Physicochemical properties, including size distribution and chemical composition, were characterized. MTT and LDH assays determined optimal doses (25–100 µg/ml). Cells were exposed acutely (4–12 h) or chronically (24–72 h). Toxicity assessments included DNA damage (8OHdG ELISA), oxidative stress (CELLROX® livecell imaging), and cell cycle analysis

(FACS). Statistical analysis used ANOVA with Dunnett's post hoc and Bonferroni correction ($p < 0.05$).Transcriptomics was performed on cells exposed to 25 µg/ml UFPs. DESeq2 (FDR <0.05) identified ~2,600 differentially expressed genes, followed by gene set enrichment analysis (GSEA) and k-means clustering to explore early vs. late transcriptional trends. Nanjing UFPs caused greater reductions in cell viability and metabolic activity, rapid mitochondrial ROS generation, and DNA damage especially at higher doses and longer exposures compared to Kuopio. PCA showed distinct clustering by UFP source and exposure duration. Nanjing UFPs upregulated hypoxia, inflammatory, detoxification, and p53senescence pathways while downregulating cell cycle and DNA repair genes, all signatures of accelerated aging. Kuopio UFPs, by contrast, activated proliferation pathways despite some DNA repair impairment, suggesting a compensatory response. K-means clustering confirmed pollutant-specific early oxidative stress from Nanjing UFPs and late-stage repair deficits. This study underscores the source- and time-dependent toxicity of UFPs in aging OM cells. Nanjing UFPs pose higher risk, emphasizing the need for tighter regulations. The integrated use of transcriptomics, imaging, and functional assays provides a robust framework to assess UFP toxicity. Ongoing comparative analyses between old and young donor cells will further elucidate mechanisms and inform targeted interventions.

OC-07

The association of gene polymorphisms of proteins involved in the regulation of redox homeostasis with the development of neurological post-COVID sequelae

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Background and aim: Exposure to SARS-CoV-2 may result in infection and, in some individuals, lead to long COVID. Neurological manifestations are the most prevalent, affecting about one third of long COVID patients.

Neuroinflammation and disturbed redox homeostasis are key mechanisms in their development. Host-specific genetic variations, particularly in genes involved in oxidative stress and inflammation, may influence clinical phenotypes of long COVID. We aimed to investigate whether antioxidant gene polymorphisms are associated with neurological sequelae in long COVID patients, stratified by acute disease severity into groups with mild and severe clinical presentations. Material and methods: Neurological examination and antioxidant genetic profile (GSTM1, GSTT1, GSTA1, GSTP1 rs1695, GSTP1 rs1138272, GSTM3 rs1332018, GSTO1 rs4925, GSTO2 rs156697, GPX1 rs1050450, SOD2 rs4880 and GPX3 rs8177412) determination, were conducted on 146 COVID19 patients. Polymorphisms were determined using PCR-based methods.

Results: Fatigue was present in 91% of patients, myalgia in 32%, “brain fog” in 9%, instability in 10%, and paresthesia in 12%. Carriers of GSTT1null genotype with mild acute illness had higher risk of post-COVID myalgia (OR=2.81, 95%CI=1.02–7.76, p=0.046), while GPX1*Leu variant allele was associated with higher risk of myalgia in cases with severe acute illness (OR=4.29, CI=1.01–18.2, p=0.049). SOD2*Val variant allele was associated with a significantly reduced risk of 'brain fog' in severe cases (OR=0.06, CI=0.01–0.84, p=0.036).

Conclusion: Certain antioxidant gene variants are associated with the risk of neurological sequelae in long COVID, highlighting genetic susceptibility not only during the initial infection but also in its prolonged aftermath. These findings may contribute to individualized therapeutic strategies for both prevention and treatment of long COVID sequelae.

OC-08

Protective effects of Mulberry leaves extract on blood cells

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The white mulberry, Morus alba, is a medicinal plant widespread in the Asian subcontinent that has attracted the attention of many researchers for the abundance of phytochemicals it contains, especially in the leaves and fruits, and for its different uses. Among the bioactive compounds contained are flavonoids, phenolic acids, terpenoids, alkaloids, etc. These secondary metabolites produced by the plant possess different biological properties such as antioxidants, anti-inflammatory, antidiabetic, neurotrophic and anticancer properties [1]. The aim of the following study is to show the antioxidant action of Morus alba leaf extract and the potential protective action exerted by it on red blood cells (RBCs). The experimental design focused on the evaluation of the antioxidant properties of Morus alba extract by DPPH, FRAP, hydroxyl radical scavenging and superoxide anion and iron chelation tests. The protective effect exerted by the extract on the morphology and integrity of the erythrocyte membrane was evaluated through morphological analysis and hemolysis assays. Finally, we evaluated the potential impact of the extract on cellular oxidative status (methemoglobin concentration, lipid peroxidation levels and sulfhydryl groups) and on cell viability (caspase 3 activity and flow cytometric analysis). Our results show the antioxidant effect of the extract, and its ability to chelate iron, at all concentrations we tested (2550100 µg/ml). The action exerted by the extract on the cell membrane, evaluated by the hemolysis assay in the presence of different stressors (temperature, presence of hydrogen peroxide, and osmotic stress) showed that the leaf extract (50100 µg/ml) protects erythrocytes from cell lysis; moreover, morphological analysis shows that the extract does not alter cell morphology. The analysis of the oxidative state of the erythrocyte in the presence of the leaf extract (50100 µg/ml), did not reveal any alteration of the oxidative state of hemoglobin, and highlighted the protective effect exerted by the same against the action of AAPH 100 mM. In fact, our results showed that the extract protects erythrocytes from the oxidizing action of AAPH, reducing lipid peroxidation levels and inhibiting the oxidation of sulfhydryl groups. Finally, cell viability, assessed by caspase 3 activity and flow cytometric analysis, shows that Morus does not cause cell damage. In their entirety our results deepen the knowledge on the antioxidant action exerted by Morus alba leaf extract, and further clarify the action exerted by the extract on the oxidative state of erythrocytes and membrane integrity.

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OC-09

A novel approach for modelling oxidative stress in the retinal pigment epithelium: characterization of a DAO RPE cell line

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Oxidative stress is a major disease mechanism in retinal disorders such as inherited retinal disease and age-related macular degeneration, two leading causes of vision loss and blindness worldwide. To properly study the role of oxidative stress in retinal disease, a reliable and robust in vitro oxidative stress model is of utmost importance. Most studies add H_2O_2 to the culture medium for stress modelling; however, this does not reflect physiological conditions and leads to highly variable results. To address this, we developed and characterized a novel ARPE19 cell line stably expressing the enzyme Damino acid oxidase (DAOARPE19), which intracellularly produces H_2O_2 upon addition of its substrate Dalanine. Two cell lines were generated using lentiviral transduction, one expressing wildtype DAO and one expressing a nonfunctional, mutated DAO. DAO expression was confirmed in both cell lines using RTqPCR, Western Blot and immunostaining. Intracellular H2O2 production was induced using a range of Dalanine concentrations (3 mM – 50 mM) combined with 3Amino1,2,4triazole, a catalase inhibitor. H_2O_2 production was verified using the redox-sensitive roGFP2Orp1 probe, demonstrating increased levels after the addition of Dalanine. Live-cell imaging via the Incucyte S3 revealed that Dala concentrations over 50 mM are cytotoxic. To investigate the downstream effects of stress induction, expression of oxidative stress markers was assessed using RTqPCR (HMOX1, SOD1, NQO1, GCLM, NFE2L2, GPX8, BCL2 and CDK2) and Western Blot (NRF2). Expression of these markers increased in stress conditions compared to basal conditions (eg. HMOX1 +74%, SOD1 + 51% and GCLM +168%). Further optimization, including RNAseq
in oxidative stress and basal conditions, is currently ongoing to study underlying molecular pathways. In conclusion, this model allows to

investigate endogenously produced oxidative stress in RPE, thereby serving as an excellent disease model for many retinal diseases.

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OC-10

Exploring the role of a long noncoding RNA in the retinal pigment epithelium and the oxidative stress response, a major disease mechanism in retinal diseases

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The oxidative stress response is a major disease mechanism underlying rare inherited as well as common multifactorial retinal diseases. We identified a long noncoding RNA (lncRNA) specifically expressed in the retinal pigment epithelium (RPE), the layer supporting the photoreceptors, with a potential role in this key pathway. LncRNAs are fascinating molecules that regulate gene expression at the right time and place, yet little is known about their function in the human retina. Specifically, this RPE-specific lncRNA is upregulated in age-related macular degeneration and upon oxidative stress. We hypothesize that it is involved in normal functioning of the RPE by playing a key role in the oxidative stress response and by regulating a lysosomal flippase. This flippase is the only protein-coding gene located in the same topologically associating domain as the lncRNA that is expressed in the RPE. To investigate this link, lncRNA knockdown using gapmer antisense oligonucleotides (ASOs) was performed in ARPE19 cells, resulting in decreased expression of the protein-coding gene (RTqPCR), suggesting that it is indeed regulated by the lncRNA. Furthermore, poly(A) RNAseq was performed on these cells. In total, 2,745 genes were significantly differential expressed in ASO-treated cells compared to negative control-ASO-treated cells. Gene Ontology enrichment analysis of the upregulated differential genes revealed terms related to endosomes, oxygen levels and endoplasmic reticulum stress. Single-molecule RNA in situ hybridisation of the lncRNA revealed accumulation in RPE-cell nuclei. Finally, knockdown in basal and upon oxidative stress conditions is ongoing in ARPE19 cells differentiated to a more RPE-like phenotype, followed by RNAseq. In this gene-focused study, fundamental insights in the functionality of the lncRNA in the human RPE are acquired. Further research is ongoing to investigate the role of the lncRNA in the disease mechanism of retinal degenerative diseases.

OC-11

Establishing a Redox Profile for Aging: Biomarker-Based Insights from the ALFA Score Project

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Aging is a dynamic and gradual process which nowadays represents a worldwide concern with great social and economic impact. As aging is a continuous process and life expectancy tends to increase, there is an urgent need to design and promote healthy aging strategies, boosting research in understanding the aging process and their players as much as possible. It is well recognized the influence of oxidative stress in age-related diseases pathophysiology and, therefore, there is an interest in characterizing the redox profile of patients, adding a layer of knowledge into the design of preventive and therapeutic approaches. The Algarve Fit Ageing Score project (ALFA Score) aims to develop a tool that allows a precise assessment of the aging status and predisposition to disease development of an individual, based on its biological profile. Here we aim to establish a redox profile associated with healthy and unhealthy aging, by analyzing different oxidative stress relevant biomarkers in human plasma from two different cohorts: healthy adult individuals (< 30 years old) and elderly (>75 years old). ELISA or colorimetric kits were used to analyze the levels of lipid peroxidation markers (8-epi-PGF2α and malondialdehyde, MDA), protein carbonyl content (PCC) based on DNPH levels, nitration (3nitrotyrosine, 3NT), DNA damage (8-hydroxy-2'-deoxyguanosine, 8OHdG) and antioxidant capacity (DPPH). Our results showed a significant increase in DNA damage marker 8OHdG, 8-epi-PGF2α, 3NT and antioxidant capacity between young and old individuals. It is also observed an increase in 8OHdG levels in plasma from old male individuals, compared to female. Our findings highlight distinct redox profiles between young and elderly individuals, particularly related to oxidative DNA damage, lipid peroxidation and nitration. Additionally, the observed increase in plasma antioxidant capacity among older participants is consistent with previous reports, particularly when antioxidant enriched diets or medical interventions are in place. Overall, these results suggest that monitoring such alterations could enable the early prediction of oxidative imbalances in aging, contributing to the development of more effective strategies for promoting healthy aging.

OC-12

Vitamin C uptake rewires microglial redox state to modify Alzheimer's disease

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Vitamin C depletion is a consistent feature of the Alzheimer's disease (AD) brain, yet decades of clinical trials using dietary supplementation have consistently failed to demonstrate benefit. This longstanding paradox suggests that the critical limitation lies not in systemic availability, but in the cellular mechanisms regulating uptake and redox balance within the brain. Microglia, as central regulators of neuroinflammation and proteostasis, are highly vulnerable to redox imbalance. Here, we identify a progressive, disease associated downregulation of the sodium-dependent vitamin C transporter SVCT2 (Slc23a2) specifically in microglia as the crucial bottleneck for ascorbate utilization in AD. We hypothesized that bypassing this transport

deficiency via targeted genetic intervention could restore microglial redox homeostasis and modify disease progression, either by preventing the onset of pathology or rescuing established deficits. To test this, we selectively overexpressed SVCT2 in the microglia of 5xFAD mice, employing both preventative (pre-onset) and therapeutic (post-onset) strategies.
Enhancing SVCT2 expression successfully increased intracellular ascorbate and triggered a profound rewiring of microglial redox metabolism. This redox reprogramming established a unique, "hybrid" neuroprotective microglial phenotype, characterized by the co-expression of homeostatic markers (e.g., Cx3cr1, Csf1r) alongside select disease-associated microglia (DAM) genes (CD11c and Lpl), thereby preserving protective functions while engage and decrease the Amyloid burden. Functionally, SVCT2-driven redox restoration improved synaptic bioenergetics and significantly decreased amyloid plaque burden. Consequently, this prevented the development of synaptic dysfunction, cognitive decline and memory deficits. Remarkably, therapeutic SVCT2 overexpression initiated after disease onset successfully rescued synaptic plasticity and memory performance. Crucially, this cognitive rescue occurred despite leaving the established amyloid pathology unchanged. We found that this late-intervention effect was mediated by the redox regulation of microglial secretory pathways, highlighting that neuronal support depends critically on the functional redox state of microglia rather than amyloid clearance alone. In conclusion, our findings resolve the clinical paradox of vitamin C in AD by demonstrating that neuroprotection is determined not by systemic supplementation but by the brain's cellular uptake capacity. We establish SVCT2 as a novel redox-based, disease-modifying target and provide a strong mechanistic rationale for therapeutic strategies aimed at restoring microglial function to counteract neurodegeneration.

OC-13

Metabolic early-warning system: oxidative bursts instantly reroute glucose to safeguard NADPH

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Oxidative stress induced by H_2O_2 redirects glycolytic flux toward the pentose phosphate pathway (PPP). One proposed regulatory mechanism involves negative feedback through the tonic inhibition of glucose-6-phosphate dehydrogenase by NADPH. However, recent evidence shows that, in the presence of glucose, NADPH levels do not decrease during the first five seconds of incubation with H_2O_2. This finding is inconsistent with the negative feedback model, which requires a drop in NADPH levels. Here we propose that PPP regulation follows an anticipatory feedback phenomenon mediated by glucose metabolism, which anticipates the demand for NADPH under oxidative stress and increases its production. To test this, we employed HEK293 cells exposed to oxidative stress by exogenous H_2O_2. To measure key metabolite levels with resolution of seconds, genetically encoded fluorescent indicators were used. Our results show that H2O2 increases the rate of glucose uptake and consumption, keeping NADPH levels stable, while in the absence of glucose or when the first enzyme of the PPP is blocked, NADPH rapidly decreases. This NADPH decrease takes around six seconds after activation of glucose metabolism. Mathematical modeling ruled out the feasibility of negative feedback; instead, the data supports the presence of an anticipatory phenomenon. Altogether, these findings open new perspectives on redox homeostasis and regulatory mechanisms of PPP associated with oxidative stress in health and disease.

OC-14

Respiration without oxygen reactions driving CO_2 emission in anoxia

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While measuring oxygen (O_2) consumption to estimate mitochondrial electron transport chain (ETC) activity is well established with standardized protocols, the evaluation of citric acid cycle (CAC) activity by monitoring CO has received comparatively less attention. Although tightly interconnected, the ETC and CAC also fulfil separate functions; therefore, monitoring both is essential for understanding how these pathways are coupled under various pathological conditions. To address this, we developed a method for simultaneously measuring O_2 consumption and carbon dioxide (CO_2) production in adherent cell lines. As CO production primarily reflects CAC flux, this approach allows for a dynamic estimate of its activity, enabling real-time assessment of redox coupling between the ETC and CAC. Using this method, we observed persistent CO production in HKC8 and HepG2 cells under anoxic conditions, suggesting residual CAC activity independent of oxygen availability. Using specific substrates and inhibitors, we further explored the reactions contributing to anoxic CO production and their role in sustaining cellular metabolism in the absence of oxygen. Finally, employing ^{13}Clabeled glucose and glutamine, we demonstrate real-time flux changes from these carbon sources to CO_2, highlighting their contributions to CAC activity under dynamic conditions.

Poster Communications

PC-01

Myricetin, inhibits Trifloxystrob-ininduced apoptosis and the OMA1-DELE1-HRI ISR mitochondrial pathway

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Human exposure to pesticides is mostly linked to occupational exposure in agricultural production or residues in food. Trifloxystrobin (TFX) has a lipophilic nature, which allows it to accumulate in the brain. Therefore, it is critical to investigate effective methods for preventing or treating brain damage caused by TFX. One interesting approach is the use of natural chemicals that are frequently present in our food. According to their various structural and sterical characteristics, flavonoids are a significant subgroup of chemicals with potent antioxidant activity. We decide to assess the potential protective benefits of Myricetin, a flavonoid with a broad range of pharmacological actions, against toxicities brought on by TFX. Cell viability, cell cycle arrest and ISR activation via the OMA1-DELE1-HRI ISR pathway were all used to assess the cytotoxicity caused by this fungicide. Apoptosis was evaluated by measuring the externalization of phosphatidylserine, release of cytochrome C, Bax and caspase 3 activation, DNA fragmentation, cytoskeleton disruption, and mitochondrial transmembrane potential ($\Delta\Psi m$). Myricetin pretreatment of SHSY5Y cells two hours prior to Trifloxystrobin exposure was found to significantly increase cell survival and restore S phase DNA synthesis. Furthermore, cells pretreated with Myricetin two hours prior to exposure to TFX did not exhibit ISR activation, as seen by the lack of peIF2α phosphorylation, downstream of activating transcription factor 4 (ATF4) and proapoptotic transcription factor CEBP homology protein (CHOP). Myricetin also reduced chromatin condensation, phosphatidylserine externalization, DNA fragmentation, cytoskeleton disruption, loss of mitochondrial membrane potential, cytochrome C release, Bax and caspase 3 activation. All of these results point to myricetin as a potent natural substance that may shield cells from the cytotoxicity, ISR induction and apoptosis caused by Trifloxystrobin.

PC-02

Radioprotective Effects of Losartan on Testicular Damage Against Acute and Late Effects of Irradiation

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Testicular dysfunction is a common side effect of radiotherapy due to off-target damage. Germ cells are highly vulnerable, and while Sertoli and Leydig cells are more resistant, they are still affected, impairing spermatogenesis and steroidogenesis. With rising youth cancer rates, strategies to preserve fertility are crucial. Losartan (LOS) has shown potential to mitigate this damage. This work aimed to determine if radiotherapy induces acute and late alterations in testicular metabolism and if LOS can restore this function. Male Wistar rats (n=47, 12 weeks old) received 2.5 Gy of ionizing radiation to the scrotum (1.05 Gy/min; Varian Clinic 6EX). LOS-treated rats received 34 mg/kg twice daily by gavage for 7 days before, during and after irradiation. Animals were euthanized at 2 and 60 days postexposure, to represent acute and late effects, respectively. Reproductive organs were weighed, serum hormones assessed (ELISA), sperm parameters analyzed following World Health Organization guidelines, testicular mRNA expression quantified (qPCR) and oxidative stress markers, such as lipid peroxidation, protein carbonylation, and protein nitration (slotblot) measured. Metabolomic profiles were obtained via 1HNMR. Acute irradiation reduced seminal vesicle weight, increased FSH, and decreased sperm concentration. Late effects included reduced testicular and epididymal weight, impaired sperm quality, increased protein carbonylation, and altered metabolic profiles. LOS mitigated acute weight loss but not sperm decline. Long-term, LOS improved sperm quality,

reduced oxidative stress, and promoted adaptive metabolic responses. Irradiation-based cancer therapy causes structural and functional testicular damage and changes the testicular metabolome of rats, while LOS has the potential to be used as a radioprotector to mitigate the adverse acute and late effects of radiation on male fertility.

PC-03

Optimization of Dimedone Switch Method for detection of S-sulfhydrated proteins in human induced pluripotent stem cells derived from Down syndrome individuals

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The gene for cystathionine betasynthase (CBS), the enzyme producing hydrogen sulfide (H_2S), located on chromosome 21, is present in extra copy in Down syndrome (DS). Dysregulation of H_2S signalling has been linked to DS, but the precise underlying mechanisms are yet to be fully identified. The present study established and characterized induced pluripotent stem cell (iPSC) lines reprogrammed from peripheral blood mononuclear cells (PBMC) collected from a blood sample of a paediatric DS patient (T21) and an age-matched apparently healthy donor (Eup). We observed that T21 cells present an increase in H_2S levels, which was accompanied by a 2-fold change increase in the levels of CBS protein, in comparison to the Eup lines. Also, we propose to identify the S-sulfhydration targets of H2S in the iPSC lines. For that, using the T21 cell line we did the optimization of the assay that detects protein persulfidation described by Zivanovic et al., 2019. So, the first step involves the reaction of 4-chloro 7-nitrobenzofurazan (NBF-Cl) with all cysteines and amino groups in proteins. Next, the persulfidated cysteines were specifically labeled by Cysteine Sulfenic Acid Probe named DCP-Bio1. The NBF-Cl signal is characterized by a fluorescence signal (emission 488nm) that can be detect on SDSPAGE gels and correspond to the total protein. The persulfidated cysteines labelled by DCP-Bio1 were detected by Streptavidin-HRP substrate. Our observations showed that 5 mM NBF-Cl was insufficient to react with all cysteines and amino groups and we determined that the optimal NBF-Cl concentration during cell lysis was 10 mM. Furthermore, we successfully verified the specific detection of persulfidated cysteines, which were found in the presence of 50 µM DCP-Bio1 when compared to the untreated sample. Overall, we established the optimal conditions of the dimedone switch method in the cellular models of DS which will allow us to detect S-sulfhydrated proteins. The obtained data will allow to better understand the biology of H_2S and its contribution to the neurodevelopment impairment in Down Syndrome patients.

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PC-04

CoAlation Mediated Inhibition of p70S6K1 Under Oxidative Stress

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Background. Ribosomal protein S6 kinase 1 (p70S6K1) is a central effector of the mTOR signalling pathway, regulating cell growth, proliferation, and metabolism. Its activation is tightly controlled by multisite phosphorylation. However, its regulation under oxidative stress conditions remains poorly understood.

Methods. We investigated the posttranslational modification of p70S6K1 by coenzyme A (CoA), known as CoAlation, under oxidative stress. Mass spectrometry was used to identify CoAlation sites of p70S6K1 in HEK293/PANK1β cell line. In vitro kinase assay assessed the functional effects of CoAlation on enzymatic activity. Molecular docking and dynamics simulations explored the interaction of CoA with the kinase domain.
Results. We identified for the first time the covalent modification of p70S6K1 by coenzyme A (CoA) in response to oxidative stress, which regulates its kinase activity. The site of CoA binding (CoAlation) was mapped by mass spectrometry to cysteine 217 (Cys217), located in the kinase activation loop and only one amino acid away from the tripeptide DFG motif, which facilitates ATP-binding. Also, the CoAlation of recombinant p70S6K1 was demonstrated in vitro and was shown to inhibit its kinase activity. Our molecular docking and dynamics analysis revealed the most likely mode for CoA binding to p70S6K1. This mechanism involves the noncovalent binding of the CoA ADP moiety to the p70S6K1 nucleotide-binding pocket, positioning the CoA thiol group in close proximity to form a covalent bond with the surface-exposed Cys217 residue.
Conclusion. Our study demonstrates that CoAlation of p70S6K1 at Cys217 serves as a redox-sensitive mechanism that suppresses kinase activity during oxidative stress. This novel mode of regulation has potential therapeutic implications for diseases involving aberrant S6K1 activity, such as cancer and metabolic disorders by developing novel inhibitors.
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PC-05

PTP1B Inhibition Improves Diabetic Wound Healing by Decreasing Oxidative Stress and Modulating Immune Responses

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Background: Diabetic foot ulcers (DFUs) are a major complication of diabetes mellitus, characterized by persistent inflammation, impaired angiogenesis, and oxidative stress. Protein tyrosine phosphatase1B (PTP1B), a negative regulator of insulin signaling, is overexpressed in diabetic condition. This study aimed to investigate the therapeutic potential of PTP1B inhibition in diabetic wound healing and to elucidate the underlying mechanisms.

Methods: Diabetes was induced in male C57BL/6 mice via intraperitoneal streptozotocin (STZ) injections (50 mg/kg) for 5 consecutive days. After 6 weeks, two 6 mm full-thickness excision wounds were made on the dorsum of each mouse. Wounds were treated topically twice daily until day 3 with either vehicle (control), Trodusquemine (PTP1B inhibitor), Protoporphyrin-IX-zinc (II) (heme oxygenase1 inhibitor, HO1i), or PTP1Bi+HO1i. Wound healing was monitored for 10 days. Inflammatory macrophage profiles (M1/M2), angiogenesis (CD31), proliferation (Ki67), HO1 expression/activity, and ROS levels were assessed. In vitro, THP1 cells were differentiated into macrophages, treated with PTP1Bi, and stimulated with LPS for 24 hours under high glucose or normoglycemic conditions. Polarization, cytokine expression, ROS production, and HO1 levels were evaluated. PTP1B expression was elevated in diabetic skin. PTP1B inhibition significantly improved wound closure (day 10: 1.1±0.2% vs 7.2±1.4% in control, $p< 0.01$), reduced M1/M2 ratio, increased vascular density (147.4±5.5%) and proliferation (141.1±5.9%), and decreased ROS levels (68.1±6.1%). HO1 expression and activity were strongly upregulated (219.4±20.7% and 170.4±15.7%, respectively), and HO1 inhibition reversed the beneficial effects. In vitro, PTP1B inhibition promoted anti-inflammatory M2 polarization, suppressed IL6, IL1β, MCP1, and elevated HO1 expression.

Conclusions: These findings demonstrate that PTP1B inhibition enhances diabetic wound healing, angiogenesis and proliferation by modulating the inflammatory and oxidative microenvironment through HO1. PTP1B represents a promising therapeutic target for the treatment of chronic DFUs.

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PC-06

Identification of Snitrosylation targets contributing to pathological aggregation of alpha-synuclein in Parkinson's Disease: a proteomic study

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Parkinson´s disease (PD) is the second most prevalent neurodegenerative disorder and is characterized by motor and nonmotor symptoms. PD is classified as a synucleinopathy and is associated with the aggregation of alpha-synuclein (aSyn) and degeneration of dopaminergic neurons. Despite several players involved in aSyn aggregation, early triggers of PD are still unknown. Several proteins interact with aSyn and alter its structural features and its tendency to aggregate. Furthermore, aSyn is a target of several posttranslational modifications (PTMs), which also can influence its toxicity. Therefore, identifying the interactome and the PTMs of aSyn are key to providing insights into novel biomarkers and possible therapeutic targets. PD is associated with oxidative and nitrosative stress, which can cause oxidative posttranslational modifications in cysteine residues such as S-nitrosylation (SNO). Although, aSyn lacks cysteine residues in its structure, NO-sensitive proteins that interact with aSyn can be modified by this PTM. This project aims to identify SNO proteome in a neuronal model of PD (SHSY5Y cells) in nitrosative conditions. Cells were exposed to increasing concentrations of a nitrosating agent, S-nitrosocysteine (CysNO) during 15 minutes and then subjected to different versions of the biotin switch assay to label modified SNO proteins. We found that CysNO was able to oxidize proteins in SHSY5Y cells, in a concentration-dependent manner, when using a biotin switch assay with DTT. Additionally, a subset of these proteins was identified as being S-nitrosated by CysNO, with the biotin switch assay with ascorbate. These data show that total proteins of SHSY5Y cells are sensitive to NO and identification of the S-nitrosylated proteins interacting with aSyn will allow us to better understand both the physiological function of aSyn as well as molecular alterations associated with contribute the pathophysiology of PD and related disorders. ** The authors marked with an asterisk equally contributed to the work.*