PP II_D109

MIRNAS BOOST BREAST CANCER AGGRESSIVENESS BY REGULATING S-NITROSOGLUTATHIONE REDUCTASE (GSNOR) LEVELS

Gianmarco Matrullo [1,*], Valeria Fiorentini [1], Costanza Montagna [1], Giuseppe Filomeni [1,2,3], Salvatore Rizza [2]. [1] *Department of Biology, University of Rome "Tor Vergata", Rome, Italy;* [2] *Redox Biology, Danish Cancer Society Research Center, Copenhagen, Denmark;* [3] *Center for Healthy Aging, Copenhagen University, Copenhagen, Denmark*

*Presenting author

S-nitrosylation is the chemical addition of nitric oxide (NO) to a free thiol (-SH) of a protein's cysteine. Various S-nitrosylated proteins (PSNOs) have been found to impact cancer-related pathways such as proliferation, migration, epithelial-to-mesenchymal transition (EMT), and programmed cell death. NO and PSNOs homeostasis are controlled by several enzymes, including NO-producing enzymes such as NO-synthases (NOSs) and different denitrosylases, with S-nitrosoglutathione reductase (GSNOR) being the most characterized, regulating NO and PSNOs levels. GSNOR represents an important regulator of cellular signaling; specifically, its ablation affects DNA repair, senescence, and global transcription and is often related to different pathological conditions. Interestingly, we found GSNOR to be strongly deregulated in breast cancer (BC), suggesting an important role in this tumor, although both the mechanism of GSNOR downregulation and how this affects BC pathogenicity still need to be fully assessed. Our in silico analysis revealed that different micro RNAs that could regulate GSNOR levels are also heavily deregulated in BC and are often associated with cancer progression, suggesting that GSNOR downregulation might represent one of the mechanisms through which these miRNAs exert their pro-tumoral activity. In this work, we aim at defining the processes underlying GSNOR downregulation and its biological relevance to highlight GSNOR as a new target for BC treatments.

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PP II_E110

IDENTIFICATION OF NITRATED PROTEINS IN HUMAN THROMBI FROM ISCHEMIC CEREBROVASCULAR EVENTS

Romina Medeiros Figueredo [1,*], Elizabeth López-Achigar [1], Esperanza Morato López [2], Carlos García [2], García [2], Antonio Martinez Ruiz [3]. [1] *Unidad Bioquímica Clínica y Hematología, Departamento de Bioquímica Clínica, Facultad de Química, Universidad de la República, Montevideo, Uruguay;* [2] *Servicio de Proteómica, Centro de Biología Molecular Severo Ochoa, CSIC-UAM, Madrid, España;* [3] *Unidad de Investigación, Hospital Universitario Santa Cristina, Instituto de Investigación Sanitaria Princesa (IIS-IP), Madrid, Spain*

*Presenting author

A thrombus is formed from fibrin fibres and platelets and is crucial in the coagulation cascade. Fibrinogen converts into fibrin through enzymatic reactions. Platelets, activated in response to vessel damage, adhere and initiate coagulation forming a stabilized clot. The thrombus contains various proteins including clotting factors, which are crucial for proper formation. Understanding thrombi composition is vital for vascular disease mechanisms and therapeutic interventions. Posttranslational modifications occur during nitroxidative distress, an imbalance in reactive species and antioxidants affecting biomolecules. The addition of a nitro functional group to the ring of tyrosine residues, producing 3-nitrotyrosine, serves as a marker of nitroxidative distress found in cardiovascular and inflammatory diseases. Nitration of fibrinogen, observed in thrombotic diseases like thrombosis and stroke, alters fibrin dynamics. Ischemic stroke, an 85% prevalent type, results from decreased blood supply due to clot or endothelium thickening, triggering nitroxidative distress. Represents the second leading cause of death and a major cause of permanent disability. This work analysed thrombi from ischemic stroke patients, searching for 3-nitrotyrosine-containing proteins as potential biomarkers. Thrombi, obtained post-thrombectomy, underwent immunoprecipitation with an anti-nitrotyrosine antibody, followed by SDS-PAGE and Reverse Phase-Liquid Chromatography-Mass Spectrometry analysis in Dynamic Exclusion Mode analysis. Data were processed using the PEAKS Studio XPro search engine for peptide identification. Thrombi exhibited varied compositions in gel electrophoresis, with different protein patterns in each. Western blot analysis showed that nitrotyrosine residues and fibrinogen fragments were present. Immunoprecipitation revealed varied molecular weights in thrombi pools, suggesting nitrotyrosine-containing polypeptide capture. RP-LC-MS/MS identified nitrated proteins related to the immune system, oxygen transport, and hemostasis, expanding the range of nitrated proteins in ischemic stroke thrombi. The analysed thrombi showed significant protein content variation. Proteins enriched with an inflammatory profile post-anti-nitrotyrosine treatment suggest potential nitrated biomarkers. These findings enhance ischemic stroke understanding, offering insights into thrombi proteomic diversity and potential applications in biomarker research and clinical practice.

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PP II_E111

MODULATION OF OXIDATIVE NEUROMETABOLISM IN ISCHEMIA/REPERFUSION BY NITRITE

Cândida Dias, Cátia F. Lourenço, João Laranjinha, Ana Ledo *. Faculty of Pharmacy and Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal

*Presenting author

While nitrite is the typical byproduct of nitric oxide (•NO) autooxidation in biological systems, under some circumstances, it can be advantageous to reduce it "back" to the signaling free radical, providing a different, non-enzymatic route for the synthesis of •NO, certain conditions can favor its reduction "back" to the signaling free radical, offering an alternative non-enzymatic pathway for •NO production. In pathophysiological conditions such as ischemia/reperfusion (I/R), where low oxygen availability limits nitric oxide synthase activity, nitrite reduction to •NO may replace enzymatic production, allowing protective modulation of mitochondrial oxidative metabolism and thus reducing the impact of I/R on brain tissue.
In the current study, we used high-resolution respirometry to evaluate the effects of nitrite in an in vitro model I/R using hippocampal slices. We found that reoxygenation was accompanied by an increase in oxygen flux, a phenomenon that has been coined "oxidative burst". The amplitude of this "oxidative burst" was decreased by nitrite in a concentration-dependent manner. These results support the notion that nitrite mediates a decrease in the hyper-reduction of the electron transport system during ischemia, decreasing the accelerated oxygen consumption that characterizes the reoxygenation phase of I/R that has been associated with an increase in oxidant production. Additionally, a pilot in vivo study in which animals received a nitrate-rich diet as a strategy to increase circulating and tissue levels of nitrite also revealed that the "oxidative burst" was decreased in the nitrate-treated animals. These results may provide mechanistic support to the observation of a protective effect of nitrite in situations of brain ischemia.

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PP II_E112

DECODING THE IMPLICATIONS OF SUBCELLULAR H_2O_2 DYNAMICS IN A STROKE-ON-A-DISH MODEL USING CEREBRAL MICROVASCULAR ENDOTHELIAL CELLS

Merve Kalkan-Yazıcı *, Enes Görkem Kekeç, Melike Tuğba Üvak, Emrah Eroğlu. *Regenerative and Restorative Medicine Research Center (REMER), Research Institute for Health Sciences and Technologies (SABITA), Istanbul Medipol University, Istanbul, Türkiye*

*Presenting author

Reactive oxygen species (ROS) play a pivotal role in reperfusion injury following cerebral ischemia, the primary treatment for ischemic stroke. The intricate vascular and cellular mechanisms driving this "reperfusion injury" have proven elusive due to the lack of effective in vivo methodologies for measuring ROS. In this study, we employed Hyper7.2 and chemogenetic technologies for multiparametric manipulation of H_2O_2 levels, with the goal of elucidating subcellular redox signals in cultured brain endothelial cells. Our investigation utilized stably Hyper7-expressing human cerebral microvascular endothelial cells (hCMEC/D3) to identify key subcellular sources of H_2O_2 production, encompassing the cytosol,

mitochondria, and nucleus. A comprehensive analysis of acute intracellular H_2O_2 signals in hCMEC/D3, subjected to hypoxia/reoxygenation and glucose deprivation, was conducted. Our results revealed that up to 2 hours of hypoxia exposure at 1 kPa did not induce H_2O_2 increase in any cell compartment within CMEC cells. However, during 3-6 hours of hypoxia exposure, a substantial H_2O_2 increase was specifically identified in the cytosol. Following 24 hours of reoxygenation at 18 kPa, H_2O_2 concentrations reverted to normal basal levels. Notably, elevated H_2O_2 levels were solely detected in the cytosol, not in the mitochondria under any conditions, indicating mitochondria's resilience to hypoxic conditions even after reoxygenation. Examination of glucose variations demonstrated that high glucose (11 mM Glc) and low glucose (Glc deprivation, 5 mM) treatments for 3 hours did not induce H_2O_2 level changes. In contrast, in different cellular compartments, subjected to 3 hours of glucose starvation, exhibited a marked increase in H_2O_2 in the cytosol and nucleus, while the mitochondria remained unaffected. In conclusion, hypoxia does not induce significant H_2O_2 changes yet glucose starvation leads to a marked increase in H_2O_2 levels. This study unveils nuanced dynamics in ROS responses, providing valuable insights for potential therapeutic interventions in stroke-related oxidative damage.

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PP II_E113

REDOX AND LIPIDOMIC ANALYSIS OF HUMAN BRAIN MICROVASCULAR ENDOTHELIAL CELLS (HCMEC/D3) ADAPTED TO PHYSIOLOGICAL OXYGEN LEVELS

Fan Yang [1,*], Daniel Simon [2,3], Emrah Eroglu [4], Dag Aarsland [5], Richard C.M. Siow [1], Giovanni E. Mann [1]. [1] *King's BHF Centre of Research Excellence, School of Cardiovascular and Metabolic Medicine & Sciences, King's College London, UK;* [2] *Department of Metabolism, Digestion and Reproduction, Imperial College London, U.K.;* [3] *Rosalind Franklin Institute, UK;* [4] *Research Institute for Health Sciences & Technologies, Istanbul Medipol University, Türkiye;* [5] *Institute of Psychiatry, Psychology & Neuroscience, King's College London, UK*

*Presenting author

The majority of studies with endothelial and other cell types are conducted in standard culture incubators gassed with 5% CO_2 and room air (18 kPa O_2), well known to induce oxidative stress. Cells *in vivo* experience significantly lower O_2 levels, with brain endothelial cells exposed to ~3-7 kPa. Alterations in lipid metabolism mediated by oxidative stress play a key role in ischemic stroke and also are involved in signal transduction. This study aimed to compare the lipidomic profile and redox phenotype of human brain microvascular endothelial cells line (hCMEC/D3) adapted long-term (5 days) to hyperoxia (18 kPa O_2) or physiological normoxia (5 kPa O_2). Redox stress was significantly increased in hCMEC/D3 cells adapted to 18 kPa compared to 5 kPa O_2, as evidenced by elevated basal intracellular GSH (Fig. 1A) and sulforaphane upregulation of NRF2 regulated NQO1 expression. Lipidomic profiling was performed using Laser Desorption - Rapid Evaporative Ionisation Mass Spectrometry, a novel ambient ionization method developed for rapid, sample-preparation-free analysis of complex biological samples. Samples were fixed, washed to remove media and cells were directly analyzed. Preliminary multivariate statistical analysis revealed 87 spectral features as significantly different between hyperoxia (18 kPa) and physiological normoxia (5 kPa). As shown in Fig. 1C, these features are tentatively annotated as glycerophospholipids, specifically phosphatidylglycerol O-36:1, Phosphatidylinositol 38:4 and 38:3. In ongoing further data analysis we are linking these characteristic shifts with specific metabolic pathways. Our study highlights the critical importance of adapting cells *in vitro* to physiological O_2 levels and provides the first insights into shifts in the complex lipidome of cells exposed to different levels of oxidative stress.

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PP II_E114

PERICYTES ARE IMPLICATED IN NO-REFLOW AFTER CEREBRAL ISCHEMIA

Nikolaus Plesnila. Institute for Stroke and Dementia Research, University of Munich, Munich, Germany

Incomplete reperfusion of the microvasculature ("no-reflow") damages salvageable brain tissue after ischemic stroke. Using longitudinal in vivo 2-photon single-cell imaging we show 87% of pericytes, perivascular cells regulating microvascular flow, constrict during cerebral ischemia, and remain constricted post-reperfusion. Moreover, we reveal ischemic pericytes are fundamentally implicated in capillary no-reflow by limiting and arresting blood flow within the first 24 hours post-stroke. Despite sustaining acute membrane damage, we observe over half of all cortical pericytes survive ischemia and respond to vasoactive stimuli, upregulate unique transcriptomic profiles and replicate. Finally, we demonstrate delayed recovery of capillary diameter by ischemic pericytes after reperfusion predicts vessel reconstruction in the sub-acute phase of stroke and that pharmacological inhibition of Rho-kinase dilates constricted pericytes thereby increasing cerebral blood flow. Cumulatively, these findings demonstrate surviving cortical pericytes remain both viable and are promising therapeutic targets to counteract no-reflow after ischemic stroke.

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PP II_E115

THE RELATIONSHIP BETWEEN MITOCHONDRIAL OXIDATIVE STRESS AND HUNTINGTON'S DISEASE PROGRESSION

Esra Nur Yiğit [1,2,*], Mehmet Şerif Aydın [2], Emrah Eroğlu [2], Gürkan Öztürk [1,2]. [1] *Department of Physiology, International School of Medicine, Istanbul Medipol University, Istanbul, Türkiye;* [2] *Regenerative and Restorative Medicine Research Center (REMER), Research Institute for Health Sciences and Technologies (SABITA), Istanbul Medipol University, Istanbul, Türkiye*

*Presenting author

Huntington's Disease (HD) is an autosomal dominant neurodegenerative disease characterized by the accumulation of mutant Huntingtin (mHtt) protein, which interferes with cellular physiological processes. Although the involvement of complex processes such as excitotoxicity, mitochondrial dysfunction, oxidative stress, transcriptional dysregulation, astrocytic dysfunction, and neuroinflammation in the development of the disease is known, the underlying molecular mechanisms remain largely elusive. Numerous studies have indicated the significant role of oxidative stress in HD pathogenesis. However, due to the limited temporal resolution and invasive nature of current analysis methods, there is still insufficient knowledge about the dynamic regulation of oxidative stress. In our study, we visualized real-time oxidative stress and mitochondrial dynamics at the single-mitochondrion level in striatal neurons using an in vitro HD model with the H_2O_2 biosensor HyPerRed. We showed that mitochondrial trafficking was interrupted, and mitochondrial H_2O_2 increased during the mutant Htt protein aggregation. Time-lapse imaging revealed that the subcellular increase in mitochondrial H_2O_2 levels, dependent on mutant Htt aggregation, was correlated with the disruption of intracellular transport. Moreover, we observed oxidative stress increase at the single organelle level with high spatial and temporal resolution for the first time through genetically encoded biosensors during the formation of Huntington's aggregates. This approach provides a new perspective on understanding the role of oxidative stress in the pathogenesis of neurodegeneration in greater detail and sheds light on potential treatments that can halt or reverse disease progression.

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PP II_E116

NOVEL MIRNA TARGETS AND CHARACTERISATION OF OPTIC NERVE FUNCTION IN A MOUSE MODEL OF HYPOPERFUSION

Matthew Padgett [1], Rafael Sebastián Fort [2], Angus Brown [1], Alex Rathbone [1], Laura Rich [1,*], Sebastien Serres [1], Jennifer Cale [1], Joern R. Steinert [1], Rebecca C. Trueman [1], Federico Dajas-Bailador [1], Tracy D. Farr [1]. [1] *School of Life Sciences, University of Nottingham, UK;* [2] *Facultad de Ciencias, Universidad de la República, Uruguay*

*Presenting author

Background: Understanding biomarkers of vascular dysfunction as mediators of cognitive decline is essential for dementia research. A promising avenue of