Protein disulfide isomerase-A1 (PDIA1), a dithiol redox chaperone primarily from the endoplasmic reticulum lumen, catalyzes disulfide reduction, oxidation, and isomerization. We have shown PDIA1's diverse impact on vascular redox signaling, including roles in regulation of NADPH oxidases, cytoskeletal organization, cell migration, and vascular smooth muscle cell (VSMC) differentiation. However, underlying mechanisms governing these cellular processes are unclear. Notably, an interaction between PDIA1 and the glycolytic enzyme α-enolase (ENO1) has been reported in interactome databases, with significant conservation. Here we address the occurrence and implications of PDIA1-ENO-1 interaction. In VSMC, we confirmed the PDIA1-ENO1 interaction through confocal co-localization, co-immunoprecipitation, and proximity ligation assays (PLA). Extensive studies using biochemical assays and NMR metabolomics suggested that altering PDIA1 expression did not disrupt VSMC metabolism, implying that the PDIA1-ENO1 interaction does not involve ENO1's primary glycolytic role. Since PDIA1 exhibits an extracellular/cell surface pool, we interrogated possible roles of alternative ("moonlighting") ENO1 functions in the plasma membrane, known to mediate migration, invasion, and extracellular matrix remodeling. In fact, PLA localized the interaction predominantly in the adherent face of the membrane and invadopodia-like processes. Cell-surface ENO1 serves as a plasminogen receptor, enabling its conversion to plasmin through urokinase action. To investigate the impact of PDIA1-ENO1 interaction on plasminogen-to-plasmin conversion in VSMCs, we exposed cells (1 h) to the PDIA1 inhibitor Bepristat, the ENO1 inhibitor ENOblock or their combination. Subsequently, a chromogenic assay was conducted with plasminogen and S2251 plasmin substrate. Both PDIA1 and ENO1 inhibitors individually reduced the conversion by 27% and 32%, respectively (n=4, $p<0.0001$). Remarkably, combining the inhibitors showed no extra effect, consistent with PDIA1 and ENO1 sharing the same cellular pathway. Conversely, inducing PDIA1 overexpression through the TetON system (24 h) increased plasmin activation. These findings suggest that PDIA1-ENO1 association regulates ENO1 alternative effects and contributes to plasmin-dependent extracellular matrix remodeling.

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Development of a redox proteomics workflow to identify cell surface proteins susceptible to thiol oxidation in endothelial cells

Danielle Vileigas[1], Railmara Silva[2], Bianca Dempsey[3], Flavia Meotti[4]
[1 3 4]University of São Paulo, São Paulo, Brazil
[2]Biochemistry, Universidade de São Paulo, São Paulo, Brazil

Thiol-disulfide switches in the cell surface proteins regulate various essential biological processes, including proliferation, adhesion, migration, inflammation, and immune response. Some membrane proteins were shown to be sensitive to thiol oxidation. However, the elucidation of which extracellular proteins are targeted by oxidation and involved in the redox mechanism is so far limited. Thus, this study aimed to standardize a method for identifying cell surface thiol-containing proteins susceptible to oxidation by diamide in human umbilical vein endothelial cells using redox proteomics. The identification of oxidized proteins was based on a thiol-reactive cell-impermeable biotin label, maleimide-PEO2-biotin, and purification by affinity chromatography with monomeric avidin agarose beads. For proteomics, the protein digestion was based on the FASP method (Filter-Assisted Sample Preparation). Further, we tested three different digestion protocols, the standard protocol of the laboratory, the standard protocol adding PNGase F, and the standard protocol adding RapiGest and PNGase F. The peptides were analyzed by LC-MS/MS with label-free quantification. We found more promising results by including only the PNGase F in the digestion protocol. This protocol was more efficient regarding the number of identified proteins, which identified 2173 proteins, and presented higher efficiency in detecting extracellular proteins, including channels, receptors, enzymes, and cytoskeleton. Cells treated with diamide presented 337 proteins in higher and 350 in lower abundance than control cells. Likewise, 224 and 245 unique proteins were found in diamide *versus* control cells, respectively. Gene ontology annotation analysis enriched several terms related to cellular component, such as extracellular region (357 proteins), plasma membrane (251), cell periphery (270), and focal adhesion (71). In conclusion, this approach was useful in identifying cell surface proteins with diamide-induced thiol redox alterations, providing insights into extracellular redox signaling. Future studies are required to elucidate the role of these modifications in different biological functions, mainly in the vascular context.

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Selective activation of RAGE in neurons and astrocytes increase ROS production in NADPH oxidase

Andrey Vinokurov[1], Evgeniya Seryogina[2], Anna Kamynina[3], Andrey Y. Abramov[4]
[1 2]Cell Physiology & Pathology Laboratory of R&D Center of Biomedical Photonics, Orel State University, Orel, Russia
[3]Research Center for Molecular Mechanisms of Aging and Age-Related Diseases, Institute of Physics and Technology (National Research University), Russia
[4]UCL Queen Square Institute of Neurology, University College London, London, United Kingdom

In the number studies the role of RAGE (receptor for advanced glycation end-products) in inflammation, oxidative stress, apoptosis as well as detoxifying, cell protection in stress conditions and homeostasis maintaining was shown. This makes especially actual the understanding the importance of RAGE in cell living and death. Most of the RAGE ligands are multitarget that makes an almost unsolvable problem to determine the result of receptor activation in the experimentally registered complicated cascade of intracellular processes. Previously it was shown that oligopeptides identical to regions of RAGE V-domain can selectively activate the receptor on the membrane of brain cells. But subsequent intracellular processes remain little studied. Using methods of live cell imaging by confocal microscopy we have found that two peptides ((60-76) (peptide 1) and Ac-(60-76)-NH_2 (peptide 7)),

which are able to direct activation of RAGE, increase the rate of ROS production by NADPH oxidase in brain slices as well as in primary cortical neurons and astrocytes. This leads to a minor decrease in intracellular concentration of reduced glutathione and increase in lipid peroxidation rate that doesn't induce an oxidative stress and doesn't increase cell death comparing to control. Using of peptide 1 during incubation of neuroglial co-culture with beta-amyloid (βA) (25-35) leads to over 40% decrease of necrotic cells comparing with the experiments without RAGE activation. But the mechanism of protection which can be the result of competitive binding of βA and peptides to RAGE or direct binding of βA and peptides remains unknown and needs to be further investigated. So, ROS overproduction by RAGE activation seems to be a physiological response to pathological ligands like advanced glycation end-products or βA and the application of peptides for cell protection can be used in a future.

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Peroxiredoxin System Mediates Transcription Factor Activation in Response to Oxidative Stress

Bryce Wilson[1], Elizabeth Jose[2], Woody March-Steinman[3], Lisa Shanks[2], Andrew Paek[2]
[1 2]Molecular and Cellular Biology, The University of Arizona, USA
[3]Program in Applied Mathematics, The University of Arizona, Tucson, USA

Reactive oxygen species (ROS) are byproducts of normal metabolic processes, and at low levels they have a vital role in cell signaling. However, high levels of ROS are highly cytotoxic, causing lipid and protein oxidation, DNA damage, and damage to other cellular components. Cells have antioxidant systems in place that serve as ROS sensors and signal mediators. The peroxiredoxins (Prdxs) are one such system that reduce peroxides such as hydrogen peroxide (H_2O_2). In response to H_2O_2 stress, Prdxs directly interact with and transfer oxidizing equivalents to proteins and transcription factors to mediate downstream responses. We have seen that after H_2O_2 treatment, several TFs are activated in distinct temporal phases: FOXO1, NFAT, and RelA are activated rapidly after H_2O_2 stress (the FOXO Phase). This is followed by deactivation of the FOXO Phase TFs and subsequent activation of p53, Jun, and NRF2 (the p53 Phase). The oxidation state of 2-Cys Prdxs is directly linked to this biphasic response of TF activation, suggesting Prdxs have a central role in coordinating the temporal dynamics of this response. 2-Cys Prdxs exist in several oligomeric states cycling between decamers, dimers, and higher molecular weight structures depending on their oxidation state. Additionally, these oligomerization states are linked to their peroxidase and signaling functions. Here, we demonstrate how 2-Cys Prdx oxidation and oligomerization is associated with the biphasic TF response.

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Dynamic Regulation of MAVS Protein Function via Reversible Oxidation at Cys79 in Response to Oxidant Stimuli

Natalia Zamorano Cuervo[1], Audray Fortin[2], Alejandro Vallejo Arroyo[3], Layla Dehbidi[2], Elise Caron[4], Nathalie Grandvaux[5]
[1 2 4 5]CRCHUM - Université de Montréal, Montreal, Canada
[3]CRCHUM, Mexico

Reversible oxidation of cysteine residues has emerged as an important regulatory mechanism of cellular responses through posttranslational modifications (Cys ox-PTMs). Identification of Cys ox-PTMs in cells is crucial for deciphering the mechanisms underlying redox regulation of proteins. The mitochondrial antiviral signaling protein MAVS is one of the central hubs for the activation of the type I and III Interferons and proinflammatory cytokine production upon sensing of invading virus. Activated MAVS forms prion-like self-perpetuating fiber-like polymers. Compelling evidence supports a role of reactive oxygen species (ROS) in the regulation of MAVS-dependent pathways, but the mechanisms remain elusive. ROS govern cellular signaling through reversible Cys ox-PTMs. As these modifications are known to modulate protein structure and function, we postulated their implication in MAVS activation and polymerization. Using maleimide-derivative bioswitch methods to label Cys ox-PTMs, we found that MAVS possesses Cys that undergo oxidation upon oxidant stimulation. Further use of specific probes allowed us to identify sulfenylation as one of MAVS Cys ox-PTM. Employing non-reducing SDS-PAGE, we found that oxidative stress is sufficient to induce MAVS oligomers containing disulfides. Via mass spectrometry, we pinpointed Cys^{79} as a target of oxidation. Using CRISPR/Cas9-engineered MAVS deficient cells reconstituted with MAVSwt, or MAVS mutated on Cys^{79}, we demonstrated that oxidation of Cys^{79} attenuates MAVS activation induced by oxidative stress. Collectively, our findings support a model wherein the function of MAVS is governed by direct oxidation of specific Cys residues. This observation holds particular relevance for autoimmune and inflammatory disorders characterized by aberrant interferon responses elicited by sterile inflammatory insults, wherein oxidative stress plays a critical and distinct role.

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Acute Intracerebral Accumulation of A-Ketoisocaproic Acid Provokes Disruption of Redox Homeostasis in Brain of Neonatal Rats

Ângela Beatris Zemniaçak[1], Rafael Teixeira Ribeiro[2], Júlia Gabrieli Bender[3], Tailine Quevedo Tavares[3], Guilhian Leipnitz[3], Moacir Wajner, Alexandre Umpierrez Amaral[4]
[1]Bioquímica, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil
[2]Bioquímica, Universidade Federal do Rio Grande do Sul,