stranded breaks caused by radiation (γH2AX, con=5.99, rad=39.93, rad+apn=20.74 percent positive). *In vivo*, mouse skin, bladder, and adipose tissues were analyzed for markers of oxidative damage 2 months post-irradiation. There was twice the level of protein nitration in the skin of irradiated animals but was reduced to control levels with adiponectin treatment. Adiponectin reduced oxidative damage to irradiated bladder (8-OHdG, con=12.10, rad=45.36, rad+apn=18.37 percent positive) and fat (8-OHdG, con=20.66, rad=43.94, rad+apn=27.17 percent positive and 4-HNE, con=20.82, rad=60.55, rad+apn=27.65 percent positive) tissues by reducing DNA damage and lipid peroxidation, respectively. Adiponectin treatment ablated epidermal thickening from radiation (con=15.42, rad=98.62, rad+apn=22.87 μm) and the significant increase in percent collagen deposition levels in both skin (con=58.44, rad=75.08, rad+apn=57.00%) and bladder (con=14.65, rad=39.65, rad+apn=21.35%). We hypothesize radiation chronically damages adipose function and secretion, negating the radioprotective effects of adiponectin.

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On the cartography of redox signaling: peroxiporins as landmark doorways

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Temporal gradients of ROS arising from different cell compartments transduce redox messages along the cell. Today, it is of no discussion that these signals play paramount roles in multiple cellular pathways and the most stable species H2O2 is widely accepted as the who. Yet, we have still important gaps in our knowledge about the where and when, and, importantly due to its involvement in disease and connections with metabolism, how this essential second messenger is controlled to avoid the harmful effects intrinsic to its hormesis. Being H2O2 producers and targets physically separated by a lipid barrier, membrane transport through bilayers stands out as a fundamental piece in the puzzle of the aforesaid how. Specific peroxiporins reside in each cellular membrane, predicting that the doors to the cytosol lead to corridors that will constitute privileged routers for redox signalling. Using genetically encoded sensors either fused to these H2O2 conduits or targeted to different organelles, we have started to trace the cartography of redox signals across the cell. The strategy has revealed interorganellar crosstalks and the existence of topologically restrained regions, suggesting that the subset of targets reached depend on their localization to the fluxes emanating from these channels.

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Peroxiredoxin 2 interacts with granule stress proteins in proliferative leukemic myeloid cells

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Peroxiredoxins (Prxd) are key players in the control of redox homeostasis in the cell, both by its peroxidase activity and its role in redox signaling. We previously found that Prxd1 and Prxd2 expression drastically decreases upon myeloid cell differentiation (de Souza et al., 2019). Likewise, other groups have reported that Prxd inhibition diminishes myeloid cell proliferation. In this study we tried to understand the early events related to redox modulation and signaling that may lead to myeloid cell differentiation. Incubation of human leukemic-60 (HL-60) cells with 1 μM retinoic acid or 1.3% DMSO did not lead to significant Prxd 1 or 2 oxidation within 24h. Conversely, there was a decrease in HyPer7 probe oxidation 4h after incubation, which was accompanied by an increase in glutathione synthesis, suggesting an overall enhanced antioxidant status. A peak in Nrf-2 migration to the nucleus was found 4 and 8h after DMSO treatment, with an increase in the transcription of HO-1, δ-GCL, NQO-1. Paradoxically, the knockdown of Prxd 2 did not promote any differentiation, indicating that Prxd 2 suppression is a consequence rather than a cause for HL-60 differentiation. Immunoprecipitation of Prxd 2 followed by proteomics revealed Prxd 2 interaction with proteins involved in RNA processing and formation of stress granules, such as G3BP1, eIF4a and eEF1A. Interaction between G3BP1 and Prx2 was further confirmed by western blot and cell image. It occurs in naïve HL-60 and HeLa cells, but it is even more evident in stress granules induced by sodium arsenite. These results suggest that Prxd might be important to maintain cell homeostasis depending on RNA metabolism and stress granules formation.

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Genome-wide case-control association study identifies a locus connecting NRF2-ARE regulation and selenium deficiency

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Genetic variants may influence selenium (Se) status leading to health effects related to sub-clinical deficiency. Thus, we aimed to identify single nucleotide polymorphisms (SNPs) associated with serum Se deficiency in Brazilian descendants. In the present study, 328 adults, of both sexes, in all classes of Body Mass Index, clinically stable, were recruited at Ribeirão Preto Medical School at the University of São Paulo, Brazil. Genotyping arrays were combined to yield 90,937 SNPs. R packages 'snpReady' version 0.9.6 (CRAN) and 'impute' version 1.68 (Bioconductor) were applied to quality control and imputation. To identify SNPs potentially associated with Se deficiency, we used a case-control design to perform an extended version of a traditional mixed model for genome-wide association analysis (GWAS) and the maximum-statistical analysis to reduce the genetic model misspecification. Functions implemented in package 'SNPassoc 2.0.11' (CRAN) were applied. The Search Tool for the Retrieval of Interacting Genes (STRING - version 11.5) was used to interrogate known protein–protein interaction networks (PPI). Our analyses identified the SNP rs425664 in 16q23.2, located in the intronic region of the MAF bZIP transcription factor (MAF). Interestingly, the post-GWAS PPI analysis indicated that MAF, as well as Kelch-like ECH-associated protein 1 (Keap1) and Nuclear factor erythroid 2-related factor 2 (NFE2L2) genes, are involved in the nuclear factor erythroid 2 p45-related factor 2-antioxidant-responsive elements (NRF2-ARE) regulation (WP4357) (FDR = 7.70e-05). Remarkably, the expression of at least four selenoproteins involved in redox regulation, namely, glutathione peroxidase 2 and 3 (GPX2 and GPX3) and thioredoxin reductase 1 and 2 (TXNRD1 and TXNRD2), is controlled by NRF2. In conclusion, our results suggested that NRF2 dysregulation could impair selenoproteins homeostasis, causing serum Se deficiency despite an adequate Se intake. These findings could be another piece of evidence pointing to the close interdependence of NRF2-regulated and Se-dependent antioxidant systems.

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Spatial-temporal detection of lipoperoxides with a genetically encoded biosensor targeted to different sub-cellular regions

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Lipoperoxides (LOOH) are reactive oxygen species produced by the oxidation of polyunsaturated fatty acids. These species play physiological roles in cell signalling, but their overproduction or inefficient withdrawal can be detrimental for cell viability and are at the genesis of several acute and chronic diseases. Despite their clinical relevance, there are not fluorescent probes allowing their non-destructive, specific and dynamic detection in cells.

With the aim to address this issue, we have developed a genetically encoded biosensor based on a fusion between a trypanosomatid´s peroxidase with high efficiency for reducing LOOH and a redox-sensitive green fluorescent protein (roGFP2).

Under in vitro conditions, the recombinant biosensor displayed a 2- to 3-orders of magnitude higher efficiency for reducing LOOH derived from linoleic acid (9(S)-HpODE) and arachidonic acid (15(S)-HpETE) than for H_2O_2. The catalytic rates for each step of the full redox cycle of the biosensor were determined by stopped-flow or conventional kinetic assays. Expressed in the cytosol of different eukaryotic cells equipped with different redox systems (Leishmania tarentolae and human hepatocytes), the biosensor responded with high sensitivity and in a reversible fashion to physiological concentrations of different LOOH added exogenously or generated endogenously. In the mammalian cell line, concurrent experiments performed with the H_2O_2 biosensor HyPer confirmed the selectivity of our biosensor for LOOH. Furthermore, the biosensor was successfully expressed in the nucleus, mitochondrial matrix and cell membrane of hepatocytes (localization confirmed by confocal microscopy using organelle and plasma membrane-specific dyes). Live cell imaging studies revealed that the magnitude and kinetics of biosensor oxidation upon exogenous LOOH stimuli were subcellular localization specific. This suggests the presence of compartment-specific redox barriers against LOOH in cells.

Based on its performance, our LOOH biosensor offers great potential for addressing questions concerning LOOH metabolism and related drug discovery campaigns in various pathophysiological cell models.

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