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Impact of PGC-1α On the Topology and Rate of Superoxide Production by the Mitochondrial Electron Transport Chain

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Reactive oxygen species (ROS) play an important role in normal signaling events and excessive ROS is associated with many pathological conditions. the amount of ROS in cells is dependent on both the production of ROS by the mitochondrial electron transport chain and removal by ROS detoxifying enzymes. the peroxisome proliferator-activated receptor γ coactivator 1α (PGC-1α) is a master regulator of mitochondrial functions and a key regulator of the ROS detoxifying program. However, the impact of PGC-1α on the topology and rate of superoxide production by the mitochondrial electron transport chain is not known. We report here using mitochondria from muscle creatine kinase (mck)-PGC-1α transgenic mice that PGC-1α does not impact the topology of ROS production, but increases the capacity of complexes I and III to generate ROS. These changes are associated with increased mitochondrial respiration and content of respiratory chain complexes. When normalizing ROS production to mitochondrial respiration, we find that PGC-1α preserves the percentage of free radical leak by the electron transport chain. Together, these data demonstrate that PGC-1α regulates the intrinsic properties of mitochondria in such a way as to preserve a tight coupling between mitochondrial respiration and ROS production.

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Effect of Lipopolysaccharide and UV-A On the ROS Generation and Cell Viability of Macrophages

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UV-A irradiation have long wavelength (320-400 nm) that can cause premature skin photoaging as well as malignant melanoma. the mechanisms induced by UV-A affects gene expression in human skin cells. Bacterial lipopolysaccharides (LPS) are potent activators of NF-kB with a stimulation of the transcription in immune cells through the intracellular generation of oxidative stress. Our objective was to evaluate the influence of LPS in macrophages (J774) irradiated with UV-A light. the cells were seeded in 96 well plates ($2x10^4$cels/well) and incubates overnight (37ºC, 5% CO_2), incubated for 3 hours with and without LPS 1µg/mL followed by UV-A irradiation in the doses of 6, 12, and 36 J/cm^2 (control was kept in the dark). Cells viability was evaluated by the MTT assay. ROS generation was measured by flow cytometry using the fluorescent probe DCFDA (2′,7′-dichlorodihydrofluorescein diacetate). UVA causes a decrease in cell viability that is proportional to the light dose and to the amount of ROS generated intracellularly. the combination of LPS with UV-A irradiation increased the generation of ROS in three times compared to the dark control and two fold compared with samples treated only with UVA. However, LPS+UVA did not cause a decrease in cell viability compared with cell treated only with UVA, showing a disconnection between ROS content and cell viability. It is likely that LPS induced the activation of several enzymatic redox routes that were able to activate the oxidation of DCFDA without exposing cell to an extra load of ROS. This experimental model is helpful to understand the multiple roles of redox misbalance and inflammatory response induced by UVA. Financial support: FAPESP.

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Hypothiocyanous Acid Modulates Cellular Function Via the Generation of Reversible and Irreversible Thiol Oxidation Products

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Macrophages recruited during inflammatory events release myeloperoxidase (MPO), an enzyme that forms oxidants including hypothiocyanous acid (HOSCN). the reactivity of HOSCN with mammalian cells is not well characterised, though protein thiol groups are known to be important targets. This is important as thiocyanate is the favoured substrate for MPO, and thus significant amounts of HOSCN are produced under physiological conditions. This study explores the mechanisms associated with HOSCN thiol oxidation observed in cellular systems.

Exposure of macrophage cells to HOSCN resulted in the selective targeting of thiol containing proteins. Proteomic experiments using the thiol-specific probe (IAF) provided evidence for the reversible modification of a number of critical cellular enzymes, including creatine kinase (CK) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Additionally, HOSCN-mediated thiol modification induced enzyme inactivation.

Exposure of isolated GAPDH and CK to HOSCN also induced thiol loss and enzyme inactivation. LCMS peptide mass mapping studies showed that the active site thiol residue was particularly sensitive to oxidation in each case. At low HOSCN doses (< 5-fold molar excess) enzyme inhibition was reversible, due to the formation of sulfenyl thiocyanate and sulfenic acid species, whilst higher doses of HOSCN resulted in irreversible enzyme inactivation and the formation of other oxygenated Cys products, including sulfinic and sulfonic acids. the formation of protein sulfenic acid derivatives was also seen in intact cells upon exposure to HOSCN, using the probe DAz-2.

This study provides the first specific evidence for formation of protein-bound sulfenic acids in mammalian cells following HOSCN exposure. This highlights a potentially novel role of HOSCN in modulating redox signalling pathways, which may be important in inflammatory disease progression.

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ASK1-p38MAPK is a New Signaling Cascade in Cardiac Myogenic Differentiation

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Myogenic differentiation is a multi-step process in which mononucleated myoblasts proliferate, then withdraw from the cell cycle, and finally differentiate and fuse to form multinucleated mature muscle fibers. p38 MAPK is a core signaling molecule in myogenic differentiation. the activation of p38 MAPK is required for myogenic differentiation; however, the mechanism for this activation remains undefined. Apoptosis signal-regulating kinase

1 (ASK1) also known as mitogen-activated protein kinase kinase kinase 5 (MAP3K5) is a member of the MAP3K family and, therefore, a part of the MAPK pathway.
Here, we reported that TNFα was significantly released from H9c2 cardiac myoblast in differentiation medium. Furthermore, the oxidant H2O2 acted as a messenger in the TNFα signaling pathway to disrupt the complex of ASK1–Trx, which was followed by the activation of ASK1 which is stimulated MKK3/6–p38MAPK signaling cascade to induce specific myogenic differentiation. in addition, exogenous TNFα added to the medium at physiological levels enhanced the ASK1–p38 MAPK signaling pathway through the increased generation of H_2O_2.
These results indicate that ASK1 is a new intracellular regulator of activation of the p38 MAPK in cardiac myogenic differentiation.

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Inhibition of Glycolysis Exacerbates 4-Hydroxynonenal-Induced Mitochondrial Dysfunction and Apoptosis

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Oxidative stress, a prevalent feature among neurodegenerative diseases, can increase mitochondrial dysfunction, eventually resulting in cell death by apoptosis or autophagy. 4-hydroxynonenal (HNE), a lipid peroxidation product that is formed during oxidative stress, accumulates in neurodegenerative diseases, and can induce the formation of other reactive species. Our lab is currently investigating how HNE impacts the cellular bioenergetics and cell survival of differentiated SH-SY5Y neuroblastoma cells. We have previously demonstrated that increasing concentrations of HNE decreases basal mitochondrial oxygen consumption, reserve capacity, and glycolytic output, leading to cell death after a 16 hour treatment. Since mitochondrial function depends on metabolic intermediates generated by glycolysis, we wanted to investigate how the interplay between glycolytic and mitochondrial energy production affects cellular susceptibility to HNE. in the current study we found that HNE induces both apoptosis, as evidenced by caspase-3 activation, and autophagy, as evidenced by LC3-I to LC3-II conversion. We blocked glycolysis using 2-Deoxyglucose (2-DG), and found that inhibition of glycolysis exacerbated mitochondrial dysfunction as early as 2 hours after HNE addition and exacerbated cell death after 16 hours of HNE treatment. Furthermore, we found that 2-DG treatment did not change the levels of the autophagy-lysosomal pathway proteins, including p62, LC3 and cathepsin D, but increased class III PI3K and Bcl-2 protein levels, and exacerbated caspase-3 activation in response to HNE. We are further investigating this phenomenon using inhibitors of autophagy and apoptosis to define the molecular pathways that lead to cell death upon 2-DG inhibition of glycolysis both in the presence and absence of HNE. This study may help to determine molecular mechanisms of cell death occurring in the brain in response to both oxidative stress and alterations in metabolism.

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A Novel Approach for Detection of Superoxide-Dependent 2-Hydroxyethidium Fluorescence Using Lambda Mode Confocal Microscopy

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Detection of superoxide ($O_2^{\bullet-}$) by hydroethidine (HE) using fluorescence microscopy techniques has been directly challenged. This is due to the well-defined overlap of the emission spectra of the non-specific HE oxidation product ethidium (E^+) with the specific $O_2^{\bullet-}$ oxidation product, 2-hydroxyethidium (2-OH-E^+). Conventional fluorescence microscopy techniques cannot distinguish E^+ and 2-OH-E^+ because of this spectral overlap. Despite this partial overlap, the emission maxima of 2-OH-E^+ is shifted approximately 20 nm relative to the E^+ emission maxima. We thus hypothesized that this difference could be resolved using lambda mode confocal microscopy where emission spectra are recorded for each pixel in a fluorescence micrograph. Two redox models were used to test this hypothesis: 1.) neuron-like N27 cells treated with the redox cycling agents menadione (20 μM) and 2,3-dimethoxy-1,4-napthoquinone (DMNQ, 25 μM), and 2.) RAW 264.7 macrophage-like cells treated with phorbol 12-myristate 13-acetate (PMA, 100 nM). Cells were treated with each compound for 3 h before addition of HE (10 μM) for 1 h further. Production of $O_2^{\bullet-}$ in both models was confirmed using HPLC separation of the oxidation products of HE as described previously. After washing, cells were imaged by confocal microscopy using excitation at 488 nm and emission collected every 10.7 nm from 506 to 699 nm. Images were then processed using spectral unmixing based on bona fide E^+ and 2-OH-E^+ spectra to remove overlapping aspects of the spectra in experimental images. the resulting micrographs indicate that 2-OH-E^+ is produced in the cell by menadione, DMNQ, and PMA. Furthermore, the subcellular localization of $O_2^{\bullet-}$ fluorescence can be determined. Application of this technique to $O_2^{\bullet-}$ detection in various cell culture-based experimental models will be discussed.

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EGFR Inhibition Induces IL6 Expression Via NOX4 in Head and Neck Cancer Cells

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The uncontrolled growth of head and neck squamous cell carcinoma (HNSCC) is associated with constitutive activation of signal-transducer-and-activator-of-transcription-3 (STAT3), which is believed to result from the persistent stimulation of epidermal growth factor receptors (EGFR) that are highly expressed in HNSCC cells. However, EGFR inhibitors fail to suppress STAT3 signaling, suggesting there are alternative routes for STAT3 activation. STAT3 has been reported to be activated by interleukin-6 (IL6), which plays a major role in cell proliferation and gene activation, suggesting that IL6 production blocks the anti-tumor activity of EGFR inhibitors. Previous studies in our lab have shown that inhibition of EGFR induces oxidative stress in HNSCC cells via NADPH oxidase-4 (NOX4). Given that IL6