Cardiolipin-Mediated Facilitation of Cytochrome C Tyrosine Nitration by Peroxynitrite

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family; VPO1 is highly expressed in the cardiovascular system, lung, liver, pancreas and spleen. However, functional roles of VPO1 have not been defined. In the present study, we demonstrate the capacity for VPO1 to catalyze formation of hypohalous acids, and characterize its enzymatic properties. VPO1, like MPO but unlike lactoperoxidase, is able to generate HOCl, HOBr and HOSCN in the presence of H_2O_2. Under physiological pH and concentrations of halides (100 μM KBr, 100 μM KSCN and 100 mM NaCl), VPO1 utilizes approximately 45% of hydrogen peroxide for the generation of HOBr, 35% for HOSCN, and 18% for HOCl. The specific activity of VPO1 is ~10 to 70-fold lower than that of MPO, depending on the specific substrate. These studies demonstrate that the enzymatic properties and substrate specificity of VPO1 is similar to MPO. Purified VPO1 as well as VPO1 in plasma mediate bacterial killing that is dependent on halide and H_2O_2; killing is inhibited by peroxidase inhibitors and by the H_2O_2 scavenger, catalase. Thus, VPO1, in addition to MPO, is the second member of the animal heme peroxidase family capable of generating HOCl under physiological conditions. VPO1 is likely to participate in host defense and mediate cell/tissue damage under pathological conditions.

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Synergistic Effects of Singlet Oxygen and Hydroxyl Radical in Photodynamic Therapy and Antitumor Immunity with Photostable Sulfonamide Bacteriochlorins

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The importance of reactive oxygen species (ROS) generation in biological processes and photodynamic therapy (PDT) is very well established. In PDT, ROS production results from irradiation of the sensitizer. Probably the most promising among currently studied photosensitizers are bacteriochlorins, tetrapyrrole compounds with two reduced pyrrole rings in the macrocycle. Halogenated sulfonamide derivatives studied in this work are example of such molecules characterized by strong absorption in the near infrared (λ_{max}≈750 nm, ε≈10^5 $M^{-1}cm^{-1}$), which enable deep penetration into tissue.[1-3] We have compared the cellular uptake, cytotoxicity and photodynamic activity of 4 new photostable synthetic bacteriochlorins with different halogen substituents in the ortho position of the phenyl rings. The spectroscopic and photophysical properties were determined and the relative contributions of Type I and Type II photochemical processes were studied by measurement of 1270 nm luminescence, EPR spin trapping and intracellular ROS detection. The difluorinated sulfonamide bacteriochlorin characterized by low dark toxicity and high phototoxicity was the most active sensitizer against several cancer cells and tumors. Our work shows that this phototoxicity does not correlate to that what should be expected from its absorbance and singlet oxygen quantum yield but rather to other reactive oxygen species formed during the illumination. We conclude that halogenated bacteriochlorins have great potential as novel PDT agents and phototoxicity may be mediated both by electron transfer (superoxide ion, hydroxyl radicals) and by energy transfer (singlet oxygen) but the photodynamic effect is significantly higher when mechanism with superoxide ion production is operated.[1-2] The effectiveness of PDT with this photosensitizer in stimulating the immune systems was related to the large inflammatory response.

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NF-κB Mediated Prx-2 Up Regulation Reduces ROS Level in Myoblast During Muscle Differentiation

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Aim: Many studies have reported that the generation of reactive oxygen species (ROS) increases during the differentiation of muscle-derived C2C12 cells. Peroxiredoxin-2 (Prx-2) is an abundant mammalian enzyme that protects against oxidative stress. However, the role of Prx-2 in muscle differentiation has not been investigated.

Results: In this study, we demonstrated that Prx-2 expression increases during muscle differentiation and regeneration in response to H_2O_2 only in myoblast cell lines because no increase in Prx-2 expression was observed in the NIH3T3, MEF, Chang, or HEK293 cell lines. The antioxidants, N-acetyl L-cysteine (NAC) and 4,5-dihydroxy-1,3-benzenedisulfonic acid (Tiron), both suppressed myogenesis and Prx-2 expression. Moreover, Prx-2 was up regulated at the transcriptional level by NF-κB during the differentiation of muscle-derived C2C12 cells. We also found that inhibition of phosphatidylinositol 3-kinase (PI3K) blocks NF-kB activation and suppresses Prx-2 expression. Interestingly, Prx-2 knockdown increased the expression levels of other antioxidant enzymes, including all of the other Prx family member, thioredoxin-1 (Trx-1) and catalase, but also enhanced the accumulation of endogenous ROS during muscle differentiation.

Innovation: In this study, we demonstrated for the first time that Prx-2 is up regulated during the muscle differentiation and regeneration.

Conclusion: Prx-2 is up regulated via the PI3K/NF-κB pathway and attenuates oxidative stress during muscle differentiation and regeneration.

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Cardiolipin-Mediated Facilitation of Cytochrome C Tyrosine Nitration by Peroxynitrite

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Cytochrome c is a small protein that functions as carrier in the mitochondrial respiratory chain and pro-apoptotic mediator in the cytosol. This protein can also act as a peroxidase when exposed to high doses of hydrogen peroxide. Recent papers showed that this peroxidase function can be enhanced when cytochrome c interacts with cardiolipin, a phospholipid from the inner mitochondrial membrane or after its oxidative post-translational modification by tyrosine nitration. Since mitochondria

are a key place of intracellularlly-generated peroxynitrite, the nitration of cytochrome c becomes a biological-relevant event. In this work, we studied the effect of cardiolipin on cytochrome c tyrosine nitration and free radical formation when exposed to peroxynitrite. Direct EPR studies showed the enhancement of protein-derived free radical formation when cytochrome c was exposed to peroxynitrite in presence of cardiolipin and EPR-spin trapping studies showed that the protein free radical formed is tyrosyl radical, a precursor of 3-nitrotyrosine. Detection of tyrosine nitration using different techniques such as immunochemistry, mass spectrometry and ion exchange chromatography showed that cytochrome c nitration is significantly facilitated by cardiolipin. Our results support that a structural change induced by cardiolipin in cytochrome c (i.e. weakening of the 6th coordination bond at the heme) increases tyrosine nitration yields, particularly in the heme-adjacent tyrosine 67, suggesting an iron-catalyzed event.

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Development of Chronic Wounds in Vivo: Imbalanced Redox State Coupled with Dysregulated Gene Expression

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Wound healing involves an intricate set of sequential but overlapping events that include hemostasis, controlled redox stress response, inflammation, re-epithelialization, granulation tissue formation, and remodeling. Unregulated and defective progression of one or more of these processes leads to impaired healing that often results in the development of chronic wounds. Diabetic foot, pressure and venous ulcers, and other similar chronic wounds have a large impact on health affecting ~5.7M people and costing ~ $20B/year in the US alone. Unraveling the causal mechanisms involved in the development and progression of chronic wounds requires animal models. We hypothesize that the wounds of mice defective in the gene LIGHT have redox imbalance which critically contributes to the development of chronic ulcers. Here we show that, much like in humans, the levels of H_2O_2 are high in adult LIGHT$^{-/-}$mice and these levels increase with age. However, the levels of the anti-oxidant enzymes catalase and glutathione peroxidase are not elevated, leading to wounds that are in a state of oxidative stress. The nitrosylation of proteins is also high, suggesting elevated levels of peroxynitrite anions, a highly reactive nitrogen species and nitrating agent that damages a wide array of molecules. Excess of lipid peroxidation further confirmed the deleterious effects of redox imbalance. We show the presence of crucial markers commonly seen in human chronic ulcers such as elevated levels of 6-keto prostaglandin F1α, 8-isoprostanes, 5-isoprostanes and arachidonic acid. These findings, coupled with our results on dysregulation of gene expression, lead to the genesis of impaired wounds. By selectively manipulating the wound environment, we have successfully produced the first chronic wounds in vivo; we will use this animal model to understand the mechanisms involved in chronic wound development and identify potential targets for treatment of humans.

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Structure and Organization of Mt Nucleoids in INS1E Cells Under Conditions of Increased Oxidative Stress

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Mitochondrial (mt) respiratory chain is a significant source of reactive oxygen species (ROS) and mtDNA represents a vulnerable target of highly reactive ROS as its oxidative damage may result in mutations initiating a self-accelerating oxidative stress. mtDNA is localized into nucleo-protein structures called nucleoids, which may have role in protection of mtDNA against oxidative stress. In this work we studied if increase in ROS generation by mitochondrial respiratory chain leads to changes in nucleoids morphology and distribution. Nucleoids size is below 300 nm and 3D super-resolution microscopy has to be applied for correct analysis of their morphology. We have employed fluorescence photoactivated localization microscopy (BiplaneFPALM) [1] to image nucleoids in INS1E cells treated with respiratory chain inhibitors rotenone and antimycin. To visualize nucleoids we used fusion of a photoconvertable Eos (or PSCFP2) with mt transcription factor A (*TFAM) or mt* single-stranded DNA-binding protein *(mtSSB). Mt network was imaged via* matrix-addressed PSCFP2 (or Eos). Rotenone treatment led to a fragmented state of solitary spheroid objects disintegrated from main mt network. Several nucleoids were clustered in those fragmented objects. We rarely observed singular mt fragments with a single nucleoid inside or empty fragments. Fragment reintegration back into the mt network re-established the tubular state with nearly equidistant nucleoid spacing. These two major morphological states coexisted at intermediate stages. Analyses of combinations of these morphological icons would provide a basis for future mitochondrial morphology diagnostics.

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PBDEs Congeners (BDE-209, BDE-99 and BDE-47) Decrease the Mitochondrial Membrane Potential and Induce ROS Accumulation on HEPG2 Cells

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Polybrominated diphenyl ethers (PBDEs) are a class of flame retardants with evidences of toxic potential caused by reactive oxygen species accumulation. The aim of this work was to investigate ROS accumulation caused by congeners BDE-47, BDE-99 and BDE-209 and evaluate if they could also affect mitochondrial membrane potential. The mitochondrial depolarization and ROS accumulation were measured using the fluorescent dyes TMRM and CM-H_2DCFDA, respectively. Cells were incubated at 37°C, in an atmosphere containing 5% CO_2 and 96% relative humidity for 24 h before treatment. PBDEs congeners in concentrations ranging from 0.1μM to 25μM were then incubated with the cells for 24 and 48 hours. Our results showed that PBDEs caused a significant decrease of the mitochondrial membrane potential and increase of reactive oxygen species accumulation for all tested congeners. The congeners BDE-47 and BDE-99 showed a dose-dependent effect