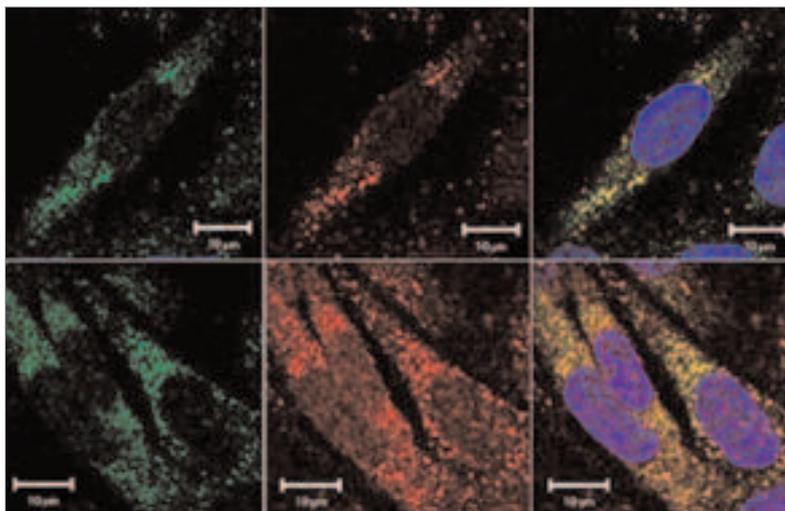


COUPLING OF ENDOPLASMIC RETICULUM STRESS TO OXIDATIVE STRESS IN VASCULAR CELLS VIA INTERACTION BETWEEN PROTEIN DISULFIDE ISOMERASE AND NAD(P)H OXIDASE: ROLE OF THIOL OXIDOREDUCTASES IN REDOX SIGNALING

Francisco Rafael Martins LAURINDO

Heart Institute (Incor)



PDI co-localizes with Nox1 and Nox4 in vascular smooth muscle cells (Janiszewski et al.: J. Biol. Chem. 2005)

Our laboratory has focused over the last several years on studying aspects of the molecular physiology of vascular redox processes. Our studies brought us to vascular NAD(P)H oxidase, a major source of reactive oxygen species (ROS). Search for putative regulatory mechanisms of this enzyme led us to the identification of protein disulfide isomerase (PDI), a thiol oxidoreductase chaperone of the endoplasmic reticulum (ER), which closely associates with vascular smooth muscle cell NAD(P)H oxidase and regulates its activation secondary to angiotensin II. Given the combination of PDI redox sensitivity with its known role

in the control of protein traffic and secretion, our results provide a novel model for understanding the NAD(P)H oxidase regulation, and consequently the cell redox status. The overall aim of our project is to further our mechanistic understanding and to explore more thoroughly some consequences of the interaction between PDI and NAD(P)H oxidase, in connection with relevant pathophysiological phenomena linked to atherosclerosis and tobacco exposure. A general hypothesis that is central to this proposal is that the interaction between PDI and NAD(P)H oxidase provides an integrative pathway for coupling between ER stress – a frequent condition in which PDI undergoes membrane traffic and may be overexpressed – with oxidative stress linked to NAD(P)H oxidase activation. The proposed investigations may reveal an innovative approach to understand why, how and where oxidative stress occurs in the vascular system. Accordingly, in addition to protocols aimed at defining basic mechanisms underlying PDI-oxidase interaction, our hypothesis will also be tested in different models that are relevant to the pathogenesis and clinical manifestations of atherosclerosis, with emphasis on vascular remodeling. Thus, we will investigate the characteristics of NAD(P)H oxidase and PDI-dependent oxidative and ER stress in models of oscillatory shear stress, vascular response to injury, and vascular and aortic valve calcification.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

We showed that protein disulfide isomerase, a dithiol disulfide oxidoreductase chaperone form in the endoplasmic reticulum (ER), displays physical and spatial interaction with the NADPH oxidase complex, assisting in its activity. This led us to assess whether oxidative stress integrates with ER stress through this pathway. Our findings indicate that: a) ER stress promotes oxidative stress; b) oxidative stress sustains both antiapoptotic and proapoptotic branches of ER stress signaling; c) ER stress promotes transcription of the Nox4 NADPH oxidase isoform, which contributes to apoptosis; d) PDI is a key integrator of oxidative and ER stress, at least in part due to its interaction with Nox4; e) vascular response to injury carries an important ER stress. These results opened many further investigations related to molecular mechanisms of PDI/NADPH oxidase interaction and the role of ER stress-associated redox processes in cell senescence. Moreover, those mechanisms integrate to ongoing and prior studies from our laboratory showing that oxidative stress is an important feature of vascular response to injury, which itself is a basic process of atherosclerosis and restenosis post-intervention.

We recently provided novel evidence indicating that a similar process occurs in degenerative aortic valve stenosis, a common disease in the elderly. Our results, both in human valves and from a rabbit model, showed important generation of oxidant species around calcifying foci in stenotic valves, by cells displaying phenotypic markers of osteoblasts/osteoclasts. Importantly, administration to rabbits of the antioxidant lipoic acid prevented valve calcification, but the unrelated antioxidant tempol led to increase in this process, indicating a complex interplay of redox events. One such level of complexity is redox compartmentation.

We have also provided several evidences for the contribution of specific microparticles derived from platelets and endothelial cells to redox processes. Also, we have now results showing a cross-talk between mitochondria, the major source of reactive species and the NADPH oxidase complex, the main dedicated source of signaling reactive species.

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Francisco Rafael Martins LAURINDO

Faculdade de Medicina
Universidade de São Paulo (USP)
Instituto do Coração - Lab. de Biologia Vascular
Av. Dr. Enéas de Carvalho Aguiar, 44
9º andar, bloco II - Cerqueira César
CEP 05403-000 - São Paulo, SP - Brasil

+55-11-3069-5185
expfrancisco@incor.usp.br