ENDOPLASMIC RETICULUM STRESSORS INDUCE PROTEIN DISULFIDE ISOMERASE UBIQUITINATION AND PHOSPHORYLATION IN VASCULAR SMOOTH MUSCLE CELLS.

Amanso AM, Santos CX, Laurindo FR
Vascular Biology Laboratory, Heart Institute, Univ. São Paulo, SP/Brazil

Ubiquitination is a reversible posttranslational protein modification and is also implicated in several nonproteolytic cellular functions. Both ubiquitination and phosphorylation regulate several functions such as gene transcription, DNA repair and replication, traffic, translocation, endocytosis. We recently described close functional/spatial association between NAD(P)H oxidase and protein disulfide isomerase (PDI), a dithiol-disulfide oxidoreductase chaperone of the endoplasmic reticulum (ER) and provided evidence that such interaction couples oxidative stress to ER stress. We hypothesized that ubiquitin/proteasome system (UPS) and PDI phosphorylation regulate such interaction. First, we showed that non-lethal concentrations of UPS inhibitors (MG132 and lactacystin) induced increase in baseline NAD(P)H oxidase activity, but a strong decrease in its response to the agonists Angiotensin-II and particularly the ER stressor Tunicamycin. We next assessed PDI ubiquitination and phosphorylation after stimulus with tunicamycin and MG132 (UPS inhibitor) in vascular smooth muscle cells. Assays of immunoprecipitation with anti-ubiquitin or anti-phosphoprotein antibodies followed by PDI western analysis revealed that PDI is ubiquitinated and serine-phosphorylated after tunicamycin (4h) and MG132 (30min) incubations. In addition, analysis of the 20S proteasome activity in presence of NADPH oxidase agonists angiotensin-II (2h) and tunicamycin (2h) induced increased proteolytic activity (ca. 40% vc control). Together, these results suggest that PDI ubiquitination and phosphorylation affect its interaction with NAD(P)H oxidase, with potentially important implications in cellular redox signaling (Supported by FAPESP, CNPq Milênio *Redoxoma*).