Evidence for a novel role of Protein Disulfide Isomerase as mediator of Rho GTPase regulation in vascular smooth muscle cells

Claro MZ, Pavanelli JC, Moretti AIS, Rodriguez AI, Fernandes DC, Laurindo FR. Vascular Biology Laboratory, Heart Institute (Incor), University of São Paulo School of Medicine.

Vascular smooth muscle cell (VSMC) proliferation and migration govern vascular development, repair to injury, and atherogenesis through redox-dependent pathways involving Nox-family NADPH oxidases. We previously described close functional and physical interaction between Nox(es) and the endoplasmic reticulum redox chaperone protein disulfide isomerase A1 (PDIA1). PDIA1 is required for angiotensin-II (AngII)-mediated Nox1 activation and platelet-derived growth factor (PDGF)-induced migration in VSMC. To assess mechanisms of PDIA1-mediated redox effects, we previously showed its strong convergence with RhoGTPases and their regulator RhoGDI through systems biology approaches using protein interactomes. Moreover, we confirmed the functional requirement of PDIA1 for Rac1 and RhoA activation and cytoskeletal structure organization. Here, we further addressed PDI-RhoGTPase convergence and hypothesized that it involves interactions between PDIA1 and RhoGDI1. We first assessed PDIA1 and RhoGDI1 immunostaining in VSMC through confocal microscopy fluorescence. Our results indicate a partial but consistent co-localization between these proteins at the perinuclear region, in vesicular-type structures distinct from pre-Golgi system, as well as in the nuclear envelope. A similar finding was obtained in cultured endothelial cells. In VSMC and endothelial cells, PDIA1 co-immunoprecipitated with RhoGDI1 in basal conditions. After PDGF incubation, association between these 2 proteins was diminished, consistent with its dynamic regulation. At the same time, the early (1-2 hour) response to PDGF involved Rac1-RhoGDI colocalization at the cell periphery, while later (4 hours) such colocalization was decreased. In parallel, PDIA1 increasingly co-localized with Rac1 at 4 hours after PDGF, suggesting transfer of Rac1 from RhoGDI to PDIA1 at this stage. In vitro, biophysical techniques such as fluorescence anisotropy further suggested a redox-dependent interaction between PDI and Rac1. These results provide further support for the dynamic interaction between PDIA1, RhoGDI and RhoGTPases in the control of VSMC function (supported by Fapesp-CEPID Redoxoma).