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THE ROLE OF OXIDATION-SENSITIVE PHOSPHOTYROSINE PHOSPHATASE, SHP-2, IN ET-1-INDUCED MITOGENIC SIGNALING PATHWAY IN CULTURED CARDIAC FIBROBLASTS

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BACKGROUND/AIMS: Endothelin-1 (ET-1) is implicated in fibroblast proliferation which results in cardiac fibrosis. Both reactive oxygen species (ROS) and epidermal growth factor receptor (EGFR) transactivation play crucial roles in the ET-1 signaling pathway. However, the connection between these two events is still unclear.

METHODS: We used rat cardiac fibroblast treated with ET-1 to investigate the link between ROS generation and EGFR transactivation. Using a Modified Malachite Green Phosphatase Assay, we further examined the involvement of oxidation-sensitive phosphotyrosine phosphatases (PTPs) in the ET-1-triggered mitogenic signaling pathway.

RESULTS: ET-1 treatment was found to stimulate the phosphorylation of EGFR and ROS generation, which were abolished by ET_A receptor antagonist BQ485. NAD(P)H oxidase inhibitor diphenyleneiodonium chloride (DPI), ROS scavenger N-acetylcysteine (NAC) and $p47^{phox}$ siRNA knockdown all inhibited the ET-1-induced EGFR transactivation. Src homology 2-containing tyrosine phosphatase (SHP-2) was shown to be associated with EGFR during ET-1 treatment by EGFR co-immunoprecipitation. We further examined the effect of ROS on oxidation-sensitive SHP-2 in cardiac fibroblasts using a Modified Malachite Green Phosphatase Assay. SHP-2 was transiently oxidized during ET-1 treatment, and this could be repressed by DPI or NAC. In SHP-2 knockdown cells, ET-1-induced phosphorylation of EGFR was dramatically elevated and was not influenced by NAC and DPI. However, this elevation was suppressed by GM6001 (a MMP inhibitor) and heparin binding (HB)-EGF neutralizing antibody.

DISCUSSION/CONCLUSIONS: We demonstrated that ET-1-ET_A-mediated ROS generation can transiently inhibit SHP-2 activity to facilitate the MMP-dependent and (HB)-EGF-stimulated EGFR transactivation in rat cardiac fibroblasts. Our findings provide new insight into the mechanism by which ET-1 activates mitogenic signaling pathways in cardiac fibroblasts, and imply a therapeutic strategy of targeting PTPs for the treatment of cardiac fibrosis.

Keywords: Epidermal growth factor receptor, Reactive oxygen species, Phosphotyrosine phosphatases