Reactive Oxygen Species Generation Is Involved in Epidermal Growth Factor Receptor Transactivation through The Transient Oxidization of SHP-2 in Endothelin-1 Signaling Pathway in Rat Cardiac Fibroblasts.

陳正憲

Chen CH;Cheng TH;Lin H;Shih NL;Chen YL;Chen YS;Cheng CF;Lian WS;Meng TC;Chiu WT;Chen JJ

摘要

Abstract

Endothelin-1 (ET-1)is implicated in fibroblast proliferation ,which results in cardiac fibrosis. Both reactive oxygen species (ROS) generation and epidermal growth factor receptor (EGFR) transactivation play critical roles in ET-1 signal transduction. In this study, we used rat cardiac fibroblasts critical treated with ET-1 to investigate the connection between ROS generation and EGFR transactivation. ET-1 treatment was found to stimulate the phosphorylation of EGFR and ROS generation, which were abolished by ETA receptor antagonist N-(N-(N-((hexahydro-1H-azepin-1-yl) carbonyl)-L-leucyl)-D- tryptophyl)-D-tryptophan (BQ485). NADPH oxidase inhibitor diphenyleneiodonium chloride (DPI), ROS scavenger N-acetyl cysteine (NAC), and p47phox small interfering RNA knockdown all inhibited the EGFR transactivation induced by ET-1. In contrast, EGFR inhibitor 4-(3 `-chloroanilino)-6,7- dimethoxyquinazoline (AG-1478) cannot inhibit intracellular ROS generation induced by ET-1. Src homology 2-containing tyrosine phosphatase (SHP-2) was shown to be associated with EGFR during ET-1 treatment by EGFR coimmunoprecipitation. ROS have been reported to transiently oxidize the catalytic cysteine of phosphotyrosine phosphatases to inhibit their activity. We examined the effect of ROS on SHP-2 in cardiac fibroblasts using a modified malachite green phosphatase assay. SHP-2 was transiently oxidized during ET-1 treatment, and this transient oxidization could be repressed by DPI or NAC treatment. In SHP-2 knockdown cells, ET-1-induced phosphorylation of EGFR was dramatically elevated and is not influenced by NAC and DPI. However, this elevation was suppressed by GM6001[a matrix metalloproteinase (MMP) inhibitor] and heparin binding(HB)-epidermal growth factor(EGF) neutralizing antibody. Our data suggest that ET-1-ETA- mediated ROS generation can transiently inhibit SHP-2 activity to facilitate the MMP-dependednt and HB-EGF- stimulated EGFR transactivation and mitogenic

signal transduction in rat cardiac fibroblasts.

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