Src homology 2-containing phosphotyrosine phosphatase regulates endothelin-1-induced epidermal growth factor receptor transactivation in rat renal tubular cell NRK-52E

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摘要

Abstract

Epidermal growth factor (EGF) and endothelin-1 (ET-1) have been shown to be involved in proliferation and autoregeneration of renal tubular cells. This study aims to investigate the regulatory mechanism of ET-1-mediated EGF receptor (EGFR) transactivation in rat renal tubular cells (NRK-52E). Exposure of NRK-52E cells to ET-1 was found to stimulate the phosphorylation of EGFR and induce reactive oxygen species (ROS) generation. Both NAD(P)H oxidase inhibitor, diphenyliodonium (DPI) and ROS scavenger N-acetylcysteine (NAC), inhibited EGFR transactivation and extracellular signal-regulated kinase (ERK) phosphorylation caused by ET-1. In contrast, blockade of EGFR by AG1478 inhibited the phosphorylation of ERK but not ROS generation following ET-1 exposure. We found that the catalytic cysteine of Src homology 2-containing phosphotyrosine phosphatase (SHP-2) was transiently oxidized by ET-1 treatment in a modified malachite green phosphatase assay. In EGFR co-immunoprecipitation, SHP-2 was also found to interact with EGFR following ET-1 treatment. In SHP-2 knockdown NRK-52E cells, ET-1-induced EGFR transactivation was dramatically elevated and not influenced by NAC. However, GM6001 (an MMP inhibitor) and heparin binding (HB)-EGF neutralizing antibody suppressed this elevation. Our data suggest that ROS-mediated oxidation of SHP-2 is essential for HB-EGF-mediated EGFR transactivation in ET-1 signaling pathway in NRK-52E cells.