Src and Epidermal Growth Factor Receptor: Novel Partners in Mediating Chronic Hypoxia–induced Pulmonary Artery Hypertension

Increased activity of the RhoA/Rho kinase (RhoA/ROCK) signaling pathway is a key feature in the development of pulmonary arterial hypertension (PAH) and the associated pulmonary vascular remodeling under various pathophysiological conditions, including chronic hypoxia. Both *in vitro* and *in vivo* studies have shown that RhoA/ROCK expression and/or activity are upregulated by chronic hypoxia, in part due to increased production of reactive oxygen species (ROS), leading to increased Ca^{2+} sensitivity in vascular smooth muscle and consequently to excessive pulmonary vasoconstriction (1–3). In an earlier publication, Norton and colleagues showed in a rat model of PAH that the enhanced Ca^{2+} sensitization and vasoconstriction of pulmonary arteries induced by chronic hypoxia were abolished not only by the inhibition of NADPH oxidase isoform 2 (NOX2) but also by an inhibitor of epidermal growth factor receptor (EGFR), demonstrating that aberrant EGFR activity may contribute to the excessive pulmonary vasoconstriction via a ROS/ROCK signaling pathway (4). It is known that EGFR can be activated by Src kinase (Src) (5) and that both Src and EGFR are involved in the development of PAH (6, 7). However, it was not known whether an Src-EGFR–dependent ROS/ROCK mechanism is involved in the pathogenesis of PAH. In this issue of the *Journal*, Norton and colleagues (pp. 61–73) report on their studies exploring the interactions among Src, EGFR, ROS, RhoA/ROCK, and matrix metalloproteinases (MMPs) in pulmonary vasoconstriction induced by chronic hypoxia and endothelin (8). They also studied the role of Src and EGFR in stretch- and endothelin-enhanced vasoconstriction via calcium sensitization through NOX-derived superoxide anions. Their major findings are that the enhanced pulmonary vasoconstriction in chronic hypoxia–exposed rats in response to pressure and endothelin-1 (ET-1) was suppressed by inhibitors of EGFR and NOX2. The increased production of superoxide caused by chronic hypoxia was also diminished by the inhibition of EGFR. Moreover, EGF caused a greater pulmonary vasoconstriction without a change in intracellular Ca^{2+} levels in rats exposed to chronic hypoxia, which was abolished by inhibition of EGFR, NOX2, and ROCK. These results indicate that enhanced pulmonary vasoconstriction in chronic hypoxia is mediated by activation of the EGFR/ROS/ROCK signaling pathway. Furthermore, they found that the enhanced pulmonary vasoconstriction after chronic hypoxia was suppressed by inhibitors of Src and type 2 MMP (MMP-2). ET-1–stimulated Src activity and the expression of MMP-2 were upregulated in these chronically hypoxic pulmonary arteries. These findings imply that Src and MMP-2 may act at points upstream of the EGFR/ROS/ROCK signaling pathway. This possibility is supported by the authors' findings that the augmented pulmonary vasoconstriction evoked by ET-1 and pressure, but not that evoked by EGF, were blunted by Src inhibition. These novel findings indicate a critical role for Src-EGFR in ROS/ROCK-mediated augmentation of pulmonary vasoconstriction, presumably due to chronic hypoxia–induced redox modulation of Src, followed by the activation of EGFR through MMP-dependent ectodomain shedding of transmembrane ligands into mature ligands that are expressed on vascular smooth muscle cells (Figure 15 in their paper).

Src kinase participates in various cellular signaling processes that are involved in the pathogenesis of pulmonary hypertension and vascular remodeling, including pathways involved in vasoconstriction, cell proliferation, and apoptosis (3, 5). Src interacts not only with membrane proteins but also with many cellular cytosolic and nuclear proteins. For instance, Src may promote pulmonary vasoconstriction through the stimulation of Ca^{2+} entry after phosphorylation of voltage-gated Ca^{2+} channels and Na^{+}/Ca^{2+} exchangers. Src may increase the sensitivity of myofilaments to Ca^{2+} through activation of RhoA/ROCK signaling via ARHGEF1, an RGS domain–containing guanine nucleotide exchange factor. In these processes the activities of Src are stimulated by hypoxia-induced production of ROS generated from NOXs and mitochondria. Activation of Src has been associated with vascular remodeling in PAH. Src can promote vascular smooth muscle proliferation by activating hypoxia-inducible factor 1 (HIF-1) or HIF-2 by inhibiting prolyl hydroxylase and von Hippel-Lindau tumor suppressor protein, thereby preventing the prolyl hydroxylation and degradation of HIF-1α or -2α. Src can phosphorylate and activate the transcription factor STAT3 (signal transducer and activator of transcription 3), and lead to increased mitogenic activity. It is important to remember that while Src activity can be stimulated by ROS, in turn, Src can enhance NOX activity through phosphorylation of the p47phox subunit and activation of Rac-1, which is required for NOX holo-enzyme assembly. Hence, a positive-feedback loop exists between Src and ROS (3, 9) (Figure 1).

The novel findings presented in this paper by Norton and colleagues (8) further delineate the mechanisms involved in chronic hypoxia–induced PAH, a situation encountered by patients with chronic obstructive pulmonary disease, alveolar hypoventilation disorders, sleep-disordered breathing, and chronic exposure to high altitude (10). In the rat model of PAH induced by monocrotaline, a pyrrolizidine alkaloid, Dahal and colleagues showed that treatment with EGFR inhibitors reduced medial wall thickening, muscularization of pulmonary arteries, and the associated right

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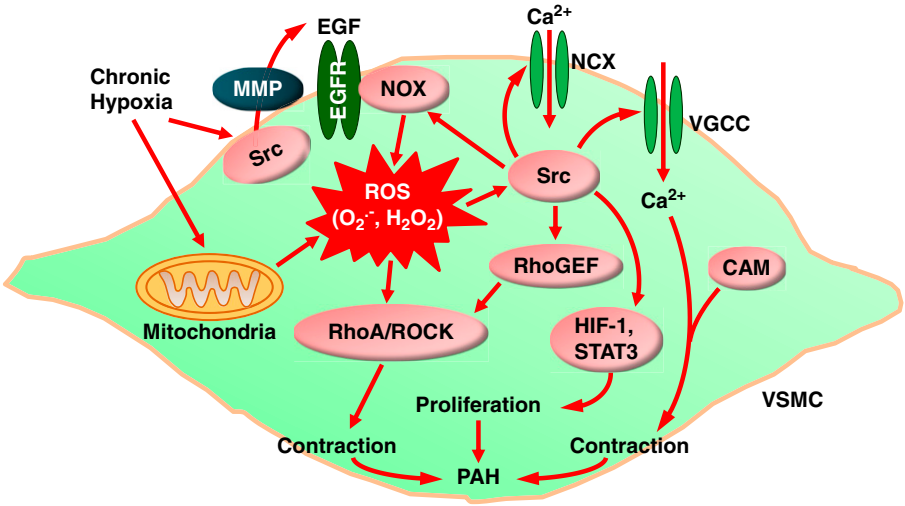

Figure 1. Possible mechanisms of Src kinase (Src) and epidermal growth factor receptor (EGFR) in the pathogenesis of pulmonary arterial hypertension (PAH). Norton and colleagues propose that chronic hypoxia stimulates the activity of Src, followed by the binding of EGF to EGFR through matrix metalloproteinase (MMP)-dependent ectodomain shedding of transmembrane ligands into mature ligands, resulting in increased activity of NADPH oxidase (NOX2) and production of superoxide anion (O_2^-) (8). Reactive oxygen species (ROS) increase the sensitivity of myofilaments to Ca^{2+} via the RhoA/Rho kinase (RhoA/ROCK) pathway, leading to excessive pulmonary vasoconstriction. It is known that chronic hypoxia can stimulate Src activity through increased ROS production from the mitochondria. Increased Src activity can also promote vasoconstriction through the RhoA/ROCK pathway via guanine nucleotide exchange factor (RhoGEF). Src may promote Ca^{2+} influx through the Na^+/Ca^{2+} exchanger (NCX) and voltage-gated Ca^{2+} channels (VGCC) to increase vasoconstriction. Src can also stimulate the proliferation of vascular smooth muscle cells (VSMCs) by suppressing the degradation of hypoxia-inducible factor 1 (HIF-1) and activating the transcription factor STAT3 (signal transducer and activator of transcription 3). It should be noted that although Src can be activated by ROS, Src also can enhance NOX activity, thereby forming a positive-feedback loop between ROS and Src (4, 9). CAM = calmodulin.

ventricular hypertrophy (11). In contrast, inhibition of EGFR did not provide any therapeutic benefit in mice with chronic hypoxia–induced PAH. In lung tissues from patients with idiopathic PAH, there was no significant change in expression of EGFR (11), but this unaltered expression of EGFR does not necessarily indicate that it is irrelevant in PAH. A recent study of patients with advanced pulmonary hypertension revealed that although the total protein levels of EGFR were unchanged in pulmonary arteries, autophosphorylation and covalent dimer formation of the receptor were enhanced, indicating increased EGFR activation (12). Norton and colleagues performed their studies using pulmonary arteries denuded of the endothelium. Signaling by both Src and EGFR occurs in pulmonary endothelial cells (13, 14). Moreover, the endothelium exerts a remarkable influence on the underlying vascular smooth muscle cells, and a complicated cross-talk occurs between these two cell types (15). Therefore, to gain a better understanding of the importance of both EGFR and Src in the pathogenesis of PAH, we need to clarify the role of the endothelium in Src and EGFR signaling in the pulmonary vasculature of individuals with PAH. ■

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