Vascular Pharmacology 58 (2013) 202–210

Contents lists available at SciVerse ScienceDirect

Vascular Pharmacology

journal homepage: www.elsevier.com/locate/vph

Rho GTPases in pulmonary vascular dysfunction

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ARTICLE INFO

Article history:
Received 3 July 2012
Received in revised form 5 September 2012
Accepted 9 September 2012

Keywords:
Pulmonary
Disease
Vascular
Rho GTPases
Therapy

ABSTRACT

Rho proteins, best known for their regulatory role in actin dynamics, stimulate a variety of processes important in the control of vascular function, including morphogenesis, migration, cell proliferation and adhesion, cell survival, gene expression, vesicle transport and microparticle formation. Rho GTPases have been implicated in several pulmonary vascular pathologies. Here we give an overview of the current knowledge of the role of Rho GTPases in vascular dysfunction, and pulmonary diseases such as pulmonary hypertension, pulmonary embolism, chronic obstructive pulmonary disease, acute lung injury and acute respiratory distress syndrome.

1. Introduction

Rho (Ras homologous) GTPases are key regulators of actin dynamics (Hall, 1998; Hall and Lalli, 2010), and play a major role in vascular processes such as endothelial permeability (Beckers et al., 2010), cell motility (Ridley, 2001), angiogenesis (Bryan and D'Amore, 2007), nitric oxide (NO) production (Takemoto et al., 2002), smooth muscle contractility (Somlyo and Somlyo, 2003), cell proliferation, differentiation and apoptosis (Pedersen and Brakebusch, 2012; Vega and Ridley, 2008). Rho proteins share approximately 30% homology with the Ras family of proteins and 80–90% homology with each other (Hall, 1998).

Rho GTPases are activated by a number of vaso-active substances such as thrombin, histamine, angiotensin II, endothelin-1 (ET-1), platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF), prostaglandin E2 via heterotrimeric G protein coupled receptors, tyrosine kinase receptors, or integrin clustering. In addition, Rho proteins are also activated by mechanical and physical stimuli such as shear stress, stretch, pressure and hypoxia (Wojciak-Stothard and Ridley, 2002; Wojciak-Stothard, 2008).

This review aims to provide an overview of the role of the best characterised Rho GTPases, RhoA, Rac1 and Cdc42, as well as the emerging role of RhoB in the regulation of pulmonary vascular function and disease (Fig. 1). Readers are also referred to other excellent recent reviews discussing the role of Rho GTPases in vascular function (Beckers et al., 2010; Connolly and Aaronson, 2011; Loirand and Pacaud, 2010; Pedersen and Brakebusch, 2012).

1.1. Regulation of Rho GTPases activity

Rho GTPases cycle between an active GTP-bound and an inactive, GDP-bound conformation. Inactive, GDP-bound Rho proteins remain in the cytosol in complex with guanine dissociation inhibitors (GDIs). Upon phosphorylation triggered by signalling mediators, GDIs dissociate from Rho GTPases, allowing them to interact with guanine nucleotide exchange factors (GEFs). GEFs activate exchange of GDP for GTP allowing Rho GTPases to interact with their downstream effectors (Jaffe and Hall, 2005). Some atypical members of this family such as RhoH or WRCH1 remain predominantly GTP-bound due either to amino-acid substitutions at residues that are crucial for GTPase activity or due to increased nucleotide exchange (Heasman and Ridley, 2008). Apart from GTP/GDP binding, Rho GTPases are also regulated through isoprenylation, carboxylmethylation, oxidation, direct phosphorylation or ubiquitination, but the extent to which these covalent modifications play a role in normal and diseased physiology is unclear.

Isoprenylation of the C-terminus of Rho GTPases enhances their binding to the cell membrane, important for the interaction with signalling effectors. Rho GTPases are prenylated by farnesyltransferase or geranylgeranyl transferase type 1 by attachment of a farnesyl (C15) or geranylgeranyl (C20) carbon chain on a C-terminal CaaX motif. Most Rho proteins including RhoA and Rac1 are modified by a geranylgeranyl moiety, whereas RhoB is unique in that it can accept either modification. Some Rho proteins, such as RhoB, are also further modified by palmitoylation (Adamson et al., 1992; Hancock et al., 1989).

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http://dx.doi.org/10.1016/j.vph.2012.09.004

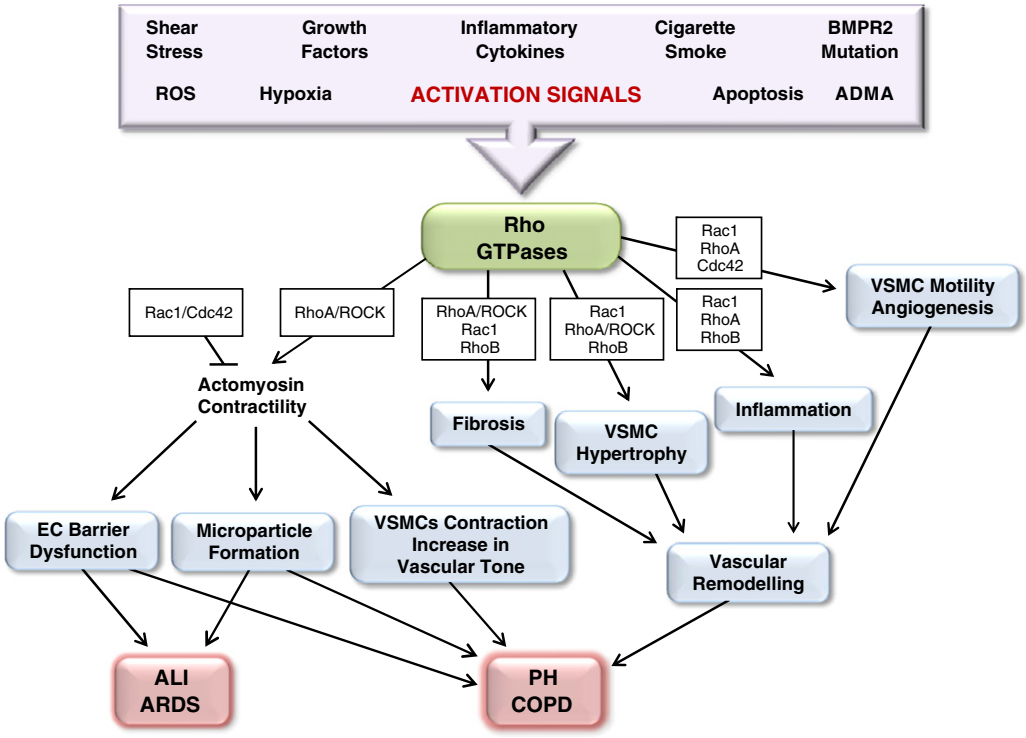

Fig. 1. Activation of Rho GTPases in the lung vasculature triggered by metabolic and environmental stresses interferes with a number of basic processes including actomyosin contractility, extracellular matrix production, cell growth, differentiation and cell motility. Resulting changes in endothelial barrier function, vascular tone and vascular remodelling contribute to development of pulmonary vascular disorders such as acute lung injury (ALI), acute respiratory distress syndrome (ARDS), pulmonary hypertension (PH) and chronic obstructive pulmonary disease (COPD).

Post-translational modifications of Rho GTPases are required for their proteasomal degradation. Carboxyl methylation of RhoA and Cdc42 has been reported to increase their half-life and geranylgeranylation increases degradation of RhoA and RhoB (Von Zee and Stubbs, 2011). Prenylation status is important in the regulation of Rho GTPases function and intracellular localization. Geranylgeranylated RhoB localises to endosomes and induces cell apoptosis (Liu et al., 2000; Wherlock et al., 2004) while farnesylated RhoB localises to the cell membrane, promotes cell growth, mediates the effects of Ras on actin cytoskeleton and activates nuclear factor kappa B (NF-κB) (Prendergast et al., 1995; Rodriguez et al., 2007; Wherlock et al., 2004).

RhoA, RhoG and Cdc42 can be regulated by phosphorylation of Ser118 by a number of kinases, including cGMP-dependent protein kinase (PKG) or cAMP-dependent protein kinase A (PKA) (Loirand et al., 2006; Guilluy et al., 2008). cGMP/PKG-mediated phosphorylation of RhoA is known to inhibit RhoA and contribute to the vasodilatory actions of NO (Loirand et al., 2006; Loirand and Pacaud, 2010; Sauzeau et al., 2003). Phosphorylation of Ser188 weakens membrane binding due to electrostatic repulsion with negatively charged phospholipids and also results in an increased affinity for GDIs. Rho-GDI is sequestered in the cytosol, disabling downstream signalling (Lang et al., 1996; Sauzeau et al., 2000). GDIα binding to RhoA-Ser188 inhibits RhoA activity but it can also promote dissociation of the Rac1–GDIα complex and cause activation of Rac1 (Rolli-Derkinderen et al., 2010). Phosphorylation additionally plays a role in regulating the levels of cellular RhoA by inhibiting ubiquitin-mediated proteasomal degradation (Rolli-Derkinderen et al., 2005).

Another kinase capable of carrying out RhoA phosphorylation on Ser188 is Ser/Thr kinase Ste20-related kinase (SLK) (Guilluy et al., 2008). This kinase acts downstream of the angiotensin II type 2 receptor (AT2R) in vascular smooth muscle cells (VSMCs). Activation of the signalling cascade involving Src homology 2 domain-containing protein-tyrosine phosphatase 1, casein kinase II and SLK is responsible for RhoA phosphorylation and inhibition of RhoA-mediated arterial contraction induced by AT2R activation.

Another possible mechanism of RhoA activation involves increased plasma serotonin (5-hydroxytryptamine, [5-HT]). Serotonin stimulates PASMC proliferation and has been associated with pulmonary arterial hypertension (PAH) and chronic obstructive pulmonary disease (COPD) (Connolly and Aaronson, 2011; Lau et al., 2012). Serotonin activates RhoA in VSMCs by promoting the association of RhoA with RhoGEF Lbc and a scaffolding protein, α-catulin (Bear et al., 2010). RhoA can also be serotonylated by transglutaminases, leading to constitutive activation (Guilluy et al., 2009).

A number of Rho proteins including RhoA, Rac1 and Cdc42 contain a redox-sensitive cysteine in their phosphoryl binding loop. Oxidation leads to guanine nucleotide release, enabling GDP–GTP cycling without assistance from GAPs. The redox-sensitive motif in RhoA [GXXXCGK(S/T)C] is distinct in that it contains two cysteine residues, and reactive oxygen species (ROS) have been shown to activate RhoA in cells through direct oxidation of these (Aghajanian et al., 2009). Conversely, exogenous oxidants can also inhibit RhoA through formation of an intramolecular disulfide bridge that prevents GTP binding (Heo et al., 2006). The effects of ROS on RhoA activity depend on ROS levels and intracellular redox potential (Lu et al., 2011). Physiological levels of ROS and high reduction potential tend to directly oxidize and activate RhoA, whereas high levels of ROS and low reduction potential tend to inhibit RhoA by formation of disulfide bridge (Nimnual et al., 2003).

2. Rho GTPases in the regulation of vascular function

The coordinated actions of Rho GTPases are required for spatio-temporal regulation of several processes important in the regulation of pulmonary and systemic vascular function, including vascular