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Small GTPases in mechanosensitive regulation of endothelial barrier

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Abstract

Alterations in vascular permeability are defining feature of diverse processes including atherosclerosis, inflammation, ischemia/reperfusion injury, and ventilator-induced lung injury. Clinical observations and experimental studies support an essential role of mechanical forces in pathophysiologic regulation of lung barrier. Accumulating data demonstrate that decreased levels of blood flow and increased cyclic stretch of lung tissues associated with lung mechanical ventilation at high tidal volumes increases vascular permeability, activates inflammatory cytokine production, alveolar flooding, leukocyte infiltration, hypoxemia, and increases morbidity and mortality. Potential synergism between pathologic mechanical stimulation and inflammatory molecules resulting in vascular leak and lung injury becomes increasingly recognized. This review will discuss a role of Rho family of small GTPases in the mechanochemical regulation of pulmonary endothelial permeability associated with ventilator induced lung injury.

Physiologic and pathologic mechanical forces in the lung

Lung is routinely exposed to mechanical forces in the forms of shear stress (SS) and cyclic stretch (CS) imposed by circulating blood and respiratory cycles.

Shear stress imposed by blood flow, mechanical strain resulting from heart propulsions and hydrostatic pressure affect all vasculature in the organism and play important role in physiological and pathological vascular responses. In contrast to vasculature from systemic circulation, additional mechanical forces resulting from respiratory cycles form unique mechanical environment experienced by pulmonary vascular endothelial cells (EC). In systemic circulation, vascular endothelium experience higher hydrostatic pressure and shear rates (15–40 dynes/cm²) (Davies, 1995; Resnick et al., 2003), and estimated amplitude of vessel distension caused by heart propulsions ranges within 5–10%. Pulmonary circulation is characterized by lower hydrostatic pressure, however shear rates in microvascular compartment may be comparable with microvasculature from systemic circulation (Ghorishi et al., 2007).

Mechanical strain experienced by endothelial cells from systemic and pulmonary circulation is a superposition of pulsatile and tonic components. Tensile stress is imposed on vascular wall

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by hydrostatic pressure counteracted by tonic contraction of vascular smooth muscle cells and elastic components. In addition, cyclic stretch is imposed by heart propulsions. Pulsatile distension of the arterial wall in systemic circulation normally does not exceed 10–12%, whereas various vasomotor reactions may change diameter of smaller caliber “resistance” arteries may reach 60% of initial diameter or more and last minutes or hours (Koller, 2002). Chronically increased blood pressure and vascular transmural stress activates vascular cell proliferation, collagen and fibronectin synthesis which results in thickening of the vascular wall as a feature of hypertension-induced vascular remodeling (Chien et al., 1998; Hu et al., 1998; O’Callaghan and Williams, 2000). In clinical settings, lung overdistention caused by mechanical ventilation at high tidal volume transmits pathologic mechanical stress to alveolar epithelium and pulmonary vasculature, which may lead to barrier dysfunction, increased inflammatory cytokine production, macrophage activation, and acute inflammation. These are hallmarks of ventilator-induced lung injury (VILI), which may culminate in pulmonary edema or acute respiratory distress syndrome.

Evaluation of physiologic and pathologic lung stretch regimen

Direct measurements of interstitial/vascular distension in the mechanically ventilated lungs are not currently available because of complexity of local distension patterns in the lung parenchyma. This is especially true during mechanical ventilation of injured lung when inflammation-induced occlusion of a part of alveolar tree results in overdistension of the functional alveoli. However, studies of alveolar epithelial cell cultures exposed to mechanical strain *in vitro* suggest that 25% increase in cell surface area corresponding to 8%–12% linear distension likely correlates with physiological levels of mechanical strain experienced by alveolar epithelium, whereas CS resulting in 37–50% increase in cell surface area corresponding to 17%–22% linear distension is relevant to pathophysiologic conditions produced by mechanical ventilation and causes progressive cell death (Tschumperlin et al., 2000). Furthermore, calculations based on animal models suggest that if lung volume increases from 40 to 100% of total lung capacity, alveolar epithelial cell basal surface area increases by 34–35% (Tschumperlin and Margulies, 1999; Wirtz and Dobbs, 2000). Cell culture models were established to reproduce VILI-associated cellular responses such as cytokine production and exacerbation of agonist-induced endothelial barrier dysfunction by high amplitude cyclic stretch observed in the injured lungs (Birukov et al., 2003; Dos Santos and Slutsky, 2000; Pugin et al., 1998; Tschumperlin et al., 2000; Vlahakis et al., 1999). Endothelial cell cultures exposed to 15%–20% cyclic stretch *in vitro* show activated VEGF expression also observed in VILI patients (Quinn et al., 2002). Because complications of mechanical ventilation develop in patients with pre-existing lung inflammation, infection or trauma, NHLBI working group emphasized the importance of two-hit animal models, which combine experimentally-induced lung inflammation and mechanical ventilation at high tidal volumes to more appropriately reflect common comorbidities and risk factors present in patients with acute lung injury (Matthay et al., 2003). Consistent with these findings, *in vitro* models of pulmonary cells exposed to pathophysiological regimen of mechanical stretch and edemagenic agonists may provide vital information about molecular mechanisms of mechanochemical regulation of lung endothelial or epithelial permeability. In addition to proinflammatory cytokine production, excessive lung distension associated with VILI directly affects blood-gas barrier and lung vascular permeability (Dos Santos and Slutsky, 2000; Oeckler and Hubmayr, 2007; Vlahakis and Hubmayr, 2000).

Magnitude-dependent effects of stretch on lung cells *in vitro*

Physiologic levels of cyclic stretch and intraluminal pressure are essential for the maintenance of vascular smooth muscle cell contractile phenotype, regulation of vascular tone and mass transport across the vessel wall. In contrast to pathologic CS, physiologic CS inhibits apoptosis

in vascular endothelium (Liu et al., 2003). Another study demonstrated that release of FGF-2, a growth factor involved in cellular reparation after injury was induced in vascular smooth muscle cells stretched at 14% and 33% elongation, but not at 5% elongation (Cheng et al., 1997). Significant increase in IL-8 production was observed in EC exposed to CS at 15% elongation, which was related to activation of pulmonary endothelium by excessive mechanical ventilation, whereas 6% CS was without effect (Okada et al., 1998). Low and high mechanical strains alternatively regulated matrix metalloproteinase-1 expression suggesting importance of specific stretch amplitudes in mechanosensitive regulation of vascular remodeling (Yang et al., 1998). Long term preconditioning at 18% CS enhanced thrombin-induced permeability in pulmonary EC monolayers even after cessation of CS stimulation (Birukov et al., 2003). In contrast, EC culturing at 5% CS decreased thrombin-induced barrier disruptive response and accelerated barrier recovery. Differences in permeability responses by EC preconditioned at high and low CS amplitudes were associated with magnitude-dependent effects of CS on expression of signaling and cytoskeletal proteins in pulmonary EC (Birukov et al., 2003). These data demonstrate distinct effects of pathologic and physiologic CS on pulmonary EC phenotypic regulation and responses to vasoactive agonists. Thus, clinical observations, animal studies and cell culture models suggest that mechanical strain exceeding 10% linear distension may be considered as pathophysiological.

In summary, although the degree of lung cell stretching is not known precisely in critically ill patients receiving mechanical ventilation, current *in vitro* studies use cyclic uniaxial strain in the range of 5%–30% elongation to mimic physiological and pathological reactions of cell stretching caused by mechanical ventilation at high tidal volumes. Available data also suggest that cyclic strain (or stretch) is more prominent factor in the endothelial cells from the lung alveolar capillaries, while shear forces may have differential effects on various portions of the vascular bed with gradient decline from the arterial endothelium in systemic circulation to the capillaries from systemic and pulmonary vascular beds.

Cytoskeletal mechanisms of EC barrier regulation

The lung endothelium forms a semi-selective barrier between circulating blood and interstitial fluid, which is dynamically regulated by a counterbalance of barrier-protective and barrier-disruptive bioactive molecules present in the circulation (Dudek and Garcia, 2001; Lum and Malik, 1996). A working model of paracellular EC barrier regulation (reviewed in (Dudek and Garcia, 2001; Mehta and Malik, 2006)) suggests that paracellular gap formation is regulated by the balance of competing contractile forces imposed by actomyosin cytoskeleton, which generate centripetal tension, and adhesive cell-cell and cell-matrix tethering forces imposed by focal adhesions and adherens junctions, which together regulate cell shape changes. Increased EC permeability in response to agonist stimulation is associated with activation of myosin light chain kinase, MAP kinases and tyrosine kinases, which trigger actomyosin cytoskeletal rearrangement, phosphorylation of regulatory myosin light chains (MLC), activation of EC contraction, destabilization of intercellular (adherens) junctions and gap formation. Previous studies by several groups suggest the important role for small GTPases Rho and Rac in the regulation cytoskeletal remodeling and EC barrier regulation (see (Bishop and Hall, 2000; Dudek and Garcia, 2001; Mehta and Malik, 2006; Wojciak-Stothard and Ridley, 2002) for review).

Mechanical forces and cell signaling in the lung

Various experimental models used to study effects of mechanical stimulation on intracellular signal transduction revealed complexity of signaling pathways activated by mechanical factors. Proposed mechanisms and molecular pathways governing mechanochemical signaling are numerous and include stretch-activated ion channels, intra- and intercellular calcium flux, G

protein-dependent and -independent kinase pathways, and the cytoskeleton that physically link matrix-bound adhesion receptors and focal adhesion complexes to the nucleus, chromosomes, and stress-responsive genes (Chien et al., 1998; Ingber, 1997; Liu et al., 1999; Maniotis et al., 1997). Mechanical strain induces activation of non-receptor tyrosine kinases p60^{Src} and focal adhesion kinase (FAK) (Liu et al., 1996; Naruse et al., 1998b), integrin-mediated signaling (Chen et al., 1999; Ikeda et al., 1999; Ingram et al., 2000; Kano et al., 2000; Naruse et al., 1998a; Schwartz and Desimone, 2008), and activates phosphorylation of focal adhesion protein, paxillin, in the lung cells (Smith et al., 1998). Shear stress- and cyclic stretch-induced activation of tyrosine kinases and MAP kinase cascades (Erk-1,2, JNK, p38) have been also reported (Chen et al., 1999; Kano et al., 2000; Li et al., 2000; Malek et al., 1999; Naruse et al., 1998a). The role of myosin light chain kinase (MLCK) in mechanical ventilation-induced lung dysfunction is less studied, however pharmacological inhibition of MLCK activity augments capillary fluid leak after experimental ventilation-induced injury (Parker, 2000). Mechanical stimulation may also activate small GTPases Rac and Rho (Birukova et al., 2006; Civelekoglu-Scholey et al., 2005; Katsumi et al., 2002; Schwartz and Desimone, 2008; Shikata et al., 2005; Tzima et al., 2002). These findings show that mechanical forces induce multiple signaling cascades in the lung.

Because the majority of putative mechanosensors and mechanotransduction pathways are stimulated by both, shear stress and stretch, it was thought that SS and CS activate similar signaling pathways; however distinct patterns of SS- and CS-activated gene expression clearly suggest distinct features of different mechanical stimuli. Differential effects of cyclic stretch and shear stress on intracellular signaling and cytoskeletal responses to mechanical stimulation have been recently addressed in our studies (Birukov et al., 2003; Birukova et al., 2006; Shikata et al., 2003), and reports by others (Katsumi et al., 2002; Tzima et al., 2002; Tzima et al., 2001). The most striking difference has been found in differential activation of small GTPases Rac and Rho by shear stress and cyclic stretch (Birukova et al., 2006; Katsumi et al., 2002; Shikata et al., 2005; Tzima et al., 2002). For example, shear stress exposure activates small GTPase Rac, which results in peripheral translocation of actin polymerization proteins and specific cortical actin remodeling (Birukov et al., 2002; Tzima et al., 2002). Other study also shows flow-induced transient increase in Rho activity prior to Rac and Cdc42 stimulation (Wojciak-Stothard and Ridley, 2003). In turn, 10–20% cyclic stretch stimulates small GTPase Rho and induces cytoskeletal remodeling distinct from shear stress (Birukov et al., 2003; Birukova et al., 2006; Shikata et al., 2005; Smith et al., 2003; Yano et al., 1996). This review will focus on the role of small GTPases in mechanochemical regulation of lung barrier function.

Small GTPases in regulation of cytoskeleton and endothelial permeability

Rho GTPases are members of the Ras superfamily of monomeric 20–30 kDa GTP-binding proteins. Ten different mammalian Rho GTPases, some with multiple isoforms, have been identified to date (Bishop and Hall, 2000). Small GTPases act as molecular switch, cycling between active GTP-bound and inactive GDP-bound state, which is regulated by guanine nucleotide exchange factors (GEFs) facilitating exchange of GDP for GTP, GTPase-activating proteins (GAPs), which increase the intrinsic rate of GTP hydrolysis by Rho GTPases, and by guanine nucleotide dissociation inhibitors (RhoGDI) which associate with inactivated Rho and Rac (Bishop and Hall, 2000; Boguski and McCormick, 1993; Zheng, 2001). The most extensively characterized members are Rho, Rac, and Cdc42, which have distinct effects on actin cytoskeleton, cell adhesions, and cell motility (Kiosses et al., 1999; Machesky and Hall, 1997; Maekawa et al., 1999; Sells et al., 1999; Timpson et al., 2001).

Among 30 potential Rho GTPase effectors identified to date (Bishop and Hall, 2000), mDia and Rho-associated kinase (Rho-kinase) appear to be required for Rho-induced assembly of stress fibers and focal adhesions (Geiger and Bershadsky, 2001; Katsumi et al., 2004). Rho-

kinase may directly catalyze MLC phosphorylation, or act indirectly via inactivation of myosin light chain phosphatase (van Nieuw Amerongen et al., 2000; Vouret-Craviari et al., 1998) by phosphorylation at Thr⁶⁹⁵, Ser⁸⁹⁴, and Thr⁸⁵⁰ (Fukata et al., 2001). Together, these mechanisms cause activation of actin polymerization, stress fiber formation, MLC phosphorylation and actomyosin-driven cell contraction resulting in EC barrier disruption.

Cdc42 controls the formation of filopodia (Etienne-Manneville and Hall, 2001; Tapon and Hall, 1997). Recent studies suggested the role of Cdc42 in the regulation of endothelial junctional permeability (Kouklis et al., 2004; Vandenbroucke et al., 2008). However, molecular mechanisms of its action on cell migration and regulation of cell monolayer barrier properties are not completely clear.

Rac initiates membrane ruffling, lamellipodia extension and formation of new adhesions cell-substrate and cell-cell adhesions. Rac effectors, WAVE, WASP, interact with Arp-2,3 complex and stimulate actin polymerization required for lamellipodia formation and cell motility (Borisy and Svitkina, 2000; Chen et al., 2000; Mullins, 2000). Another Rac effector, p21-associated kinase, or PAK1 regulates LIM-kinase mediated actin polymerization, reduction of actomyosin contraction, and stimulation of focal adhesion turnover in lamellipodia and cell ruffles (Bagrodia and Cerione, 1999; Sanders et al., 1999; Sells et al., 1999; Zhao et al., 2000). However, in other models PAK1 contributes to stress fiber formation and MLC phosphorylation (Kiosses et al., 1999). Physiologic activation of Rac by barrier-protective molecules (i.e. sphingosine-1 phosphate, oxidized phosphocholine, hepatocyte growth factor) and specific mechanical stimuli (laminar shear stress, low magnitude cyclic stretch) enhances peripheral actin cytoskeleton, induces peripheral redistribution of focal adhesions, and improves EC monolayer integrity (Birukov et al., 2002; Birukova et al., 2007a; Birukova et al., 2006; Birukova et al., 2007b; Dudek et al., 2004; Garcia et al., 2001; Mehta et al., 2005; Vouret-Craviari et al., 2002).

Thus, differential effects of Rac and Rho on endothelial cytoskeleton and permeability suggest that the balance between Rho- and Rac-mediated signaling may be a critical component of pulmonary endothelial barrier regulation in pulmonary endothelium exposed to mechanical and chemical stimulation.

Mechanical forces in regulation of small GTPases and lung endothelial barrier

Shear stress

In endothelial cells, laminar flow induces distinct patterns of cytoskeletal remodeling characterized by activation of Rac, Rac-dependent peripheral translocation of actin polymerization activator cortactin and enhancement of cortical actin cytoskeleton and peripheral focal adhesions (Birukov et al., 2002; Shikata et al., 2005; Tzima et al., 2002; Wojciak-Stothard and Ridley, 2003). Inhibition of Rac or Rac effector PAK1 activities prevented shear stress-induced endothelial orientation response (Birukov et al., 2002). Rho and Rac1 also regulated directionality of flow-induced endothelial cell movement (Wojciak-Stothard and Ridley, 2003). Shear stress causes polarization of intracellular Cdc42 activity which is required for flow-induced reorientation of microtubule organizing center toward the direction of movement leading to realignment of microtubule cytoskeleton and cell reorientation (Tzima et al., 2003). Additional data show negative effect of Rho and Rho kinase inhibition on endothelial alignment to shear stress (Tzima et al., 2002; Wojciak-Stothard and Ridley, 2003). Shear stress-induced activation of Rac signaling, enhancement of peripheral actin cytoskeleton and focal adhesion remodeling has been linked with barrier protective endothelial responses reflected by flow-induced increases in transendothelial electrical

resistance (DePaola et al., 2001; Seebach et al., 2000). However, Rac activation is not a universal mechanism of cell response to flow. For example, shear stress (5 dyn/cm²) exposure of neutrophils differentiated *in vitro* reduced Rac activity and caused retraction of pseudopods leading to decreased neutrophil adhesiveness to the luminal endothelium (Makino et al., 2005). Thus, deactivation of Rac activity by shear stress in circulating leukocytes may reflect an important homeostatic mechanism of flow-induced stabilization of leukocyte circulation.

Cyclic stretch in the 10–20% range has been shown to activate Rho in pulmonary smooth muscle cells (Smith et al., 2003), epithelium (Thomas et al., 2006) and endothelium (Birukov et al., 2003; Birukova et al., 2006; Shikata et al., 2005). It was also noted, that stretch-induced Rho activation was accompanied by reduction of basal Rac activity and inhibition of lamellipodia formation (Birukova et al., 2006; Katsumi et al., 2002). In the non-pulmonary cells, 10–15% CS also caused activation of Rac (Chaturvedi et al., 2007; Pan et al., 2005). In contrast to 10–20% stretch, 5% stretch did not cause marked Rho activation or reduction of Rac activity in endothelial cells (Birukova et al., 2006; Katsumi et al., 2002). These findings indicate that stretch-induced small GTPase signaling and cell responses may be differentially regulated by CS amplitudes and may also be cell type specific.

Activation of small GTPases by mechanical forces has been analyzed by pulldown of specific GTPases in a GTP-bound form using beads with immobilized binding domains of particular GTPase targets. Rac and Rho association with cell membrane also has been widely used as indication of GTPase activation. As alternative approach, Clark *et al.* (Clark et al., 2004) used loading cells with radioactively labeled nonhydrolyzable photoreactive GTP analogue prior to cyclic stretch stimulation. Activated GTPase in cell lysates was then detected after electrophoresis by increased incorporation of radioactive GTP analogue into specific protein bands. The identity of activated GTPases was determined by western blot with a panel of antibodies against known GTPases. This study identified small GTPase rab5 and Gα_i subunit of heterotrimeric G proteins as potentially novel stretch-activated signal proteins.

Amplitude-dependent effects of cyclic stretch on small GTPases and endothelial barrier properties

Mechanical stimuli of different origins and magnitude induce differential activation of the small GTPases Rac and Rho (Katsumi et al., 2002; Tzima et al., 2002). Importantly, differential effects of cyclic stretch amplitudes on intracellular signaling, cytoskeletal responses to mechanical stimulation and barrier regulation are becoming increasingly recognized. We have recently shown the differential effects of physiologic and pathologic magnitudes of applied cyclic stretch (CS) on agonist-induced pulmonary EC barrier disruption (Birukova et al., 2006; Shikata et al., 2005). Consistent with differential effects on monolayer integrity, 18% CS enhanced thrombin-induced Rho activation, whereas 5% CS promoted Rac activation critical for EC recovery phase. These studies suggest critical roles for amplitude-dependent cyclic stretch and Rac/Rho GTPase balance in mechanochemical regulation of the lung EC barrier. Suppression of Rac activity and lamellipodia formation in endothelial cells was observed in response to high amplitude mechanical strain (Katsumi et al., 2002). Other studies show stretch-induced activation of Rho in endothelial culture (Shikata et al., 2005; Yano et al., 1996). These data provide further support for differential effects of mechanical stimulation on Rac/Rho activation.

Rho GTPases and mechanisms of mechanotransduction

Cellular mechanisms of mechanosensing

Cell membranes, cell attachment sites, and cytoskeletal network directly experience hemodynamic forces, and most likely serve as primary mechanosensors (Davies, 1995).

However, the action of such “force receptors” is poorly characterized. Stretch-activated ion channel represents one example of such mechanosensor (Gillespie and Walker, 2001). This channel is tethered to cytoskeletal and external anchors via intracellular and extracellular linkers. Thus, mechanical forces transduced via these linkers to the channel can affect ion conductivity and activate intracellular signaling in an amplitude-dependent fashion.

Focal adhesions and mechanotransduction

Mechanical stretch from underlying capillary wall is transmitted to endothelial cells through their adhesive contacts. Engagement of integrin-containing focal adhesions and application of external force to cells may be achieved experimentally by twisting of cell-attached magnetic beads coated with integrin ligand RGD. Mechanically challenged cells increase their resistance to applied deformation and exhibit “stiffening response” (Wang et al., 1993). Focal adhesions (FA) are multi-molecular complexes consisting of more than 50 different proteins (Brown and Turner, 2004). FA form a bi-directional linkage between the actin cytoskeleton and the cell-extracellular matrix interface (Critchley, 2000) and provide additional tethering forces that help maintain endothelial cell barrier integrity. Binding to the ECM induced the attachment of integrins to intracellular actin fibers, a process in endothelial cells that stimulates phosphorylation of multiple proteins such as paxillin, GIT-2, PAK1, focal adhesion kinase (FAK), etc. (Brown and Turner, 2004; Turner et al., 2001; Zhao et al., 2000). Because integrins not only physically connect the cytoskeleton to the extracellular matrix but also function as signaling receptors, they are recognized as the important transmitters of physical forces into chemical signals (Bershadsky et al., 2003; Geiger and Bershadsky, 2001; Katsumi et al., 2004; Schwartz and Desimone, 2008). Focal adhesions have been long time considered as mechanosensors involved in cellular responses to shear stress and cyclic stretch (Romer et al., 2006). C-Src-dependent tyrosine phosphorylation of paxillin, FAK, p130Cas, as well as activation of MAP kinase signaling has been noted (Katsumi et al., 2004; Liu et al., 1996; Naruse et al., 1998b). However, exact mechanisms of stretch-induced remodeling and signaling by focal adhesions await further investigation. External mechanical forces cause remodeling of focal adhesions dependent on the nature and magnitude of applied mechanical stimulation. Mechanical strain or centripetal pulling of the cell by micropipette caused redistribution of focal adhesions, their elongation and increases in size (Riveline et al., 2001; Shikata et al., 2005). This process triggers Rho signaling leading to activation of Rho kinase-dependent increase in actomyosin contraction (Riveline et al., 2001) and signaling in the focal adhesions (Naruse et al., 1998b; Yano et al., 1996). Another Rho target, formin homology protein mDial1 is specifically involved in force-induced focal contact formation (Riveline et al., 2001).

Intercellular adhesions and mechanical signaling

Vascular endothelial specific cadherin, VE-cadherin, has been associated with endothelial responses to shear stress (Noria et al., 1999). However, its role in mechanical signaling is less obvious. VE-cadherin is shown to serve as adaptor in endothelial orientation and gene expression response to flow, where PECAM-1 served as force transducer leading to activation of signaling by VEGFR2 and PI3 kinase (Tzima et al., 2005). VE-cadherin appears to be involved in stretch-induced endothelial proliferation (Liu et al., 2007). Engagement of VE-cadherin containing adherens junction in stretch-induced Rac activation in the pulmonary endothelial cells reported in this study requires further investigation. Interestingly, stretch-induced proliferation of pulmonary vascular smooth muscle cells was associated with activation of Rho but was blocked by inhibition of both Rac and Rho GTPases (Liu et al., 2007). Thus, although published data show direct role for Rho and Rac GTPases in cell orientation, cytoskeletal remodeling, and endothelial permeability responses, the mechanisms of flow- or stretch-induced regulation of Rac and Rho remain elusive.

Role of caveoli in stretch- and flow-mediated signal transduction and Rho GTPase activation

Many cell types including endothelium contain specialized plasma membrane microdomains also referred as lipid rafts or caveolae. These cholesterol-enriched plasma microdomains (CEM) also contain sphingomyelin, gangliosides, PIP2, DAG, and a specific scaffolding protein caveolin-1 and are important sites for concentrating transmembrane receptors, and signaling proteins that transduce chemical and mechanical signals into the cell (Minshall et al., 2003; Pike, 2004). In the vascular smooth muscle cells, stretch-induced activation of FAK, Erk1/2 and Rho does not require caveolin-1 (Albinsson et al., 2008). In turn, in endothelial cells Akt activation is insensitive to stretch, but activated by shear stress in a caveolin-1-dependent fashion. These data suggest that endothelium dependent sensing of shear stress is primarily associated with caveoli-dependent Akt phosphorylation, whereas stretch sensing by vascular smooth muscle cells involves rapid MAPK and slow Rho-cofilin signaling. Exposure of mesangial cells to 10% equibiaxial cyclic mechanical strain caused Rho activation that was dependent on Rho-caveolin-1 interaction and was abrogated by disruption of caveoli using filipin or cyclodextrin treatment (Peng et al., 2007). Stretch-induced activation of both, RhoA and Rac1 through caveoli was reported in cardiomyocytes (Kawamura et al., 2003). It was suggested that in these cell type activation of RhoA and Rac1, localized in a caveolar compartment was essential for sensing externally applied force and transducing this signal to the actin cytoskeleton and Erk1/2 translocation.

Rho and Rac regulation by combined mechanochemical stimulation

Effects of combined mechanical and agonist stimulation on endothelial signaling and barrier regulation remain less explored. Pathologic stretch (18% CS) promoted thrombin-induced gap formation and delayed monolayer recovery. These effects were associated with enhancement of thrombin-induced Rho activation by pathologic CS and suppression of Rac activation at later time points required for recovery of EC monolayer integrity after thrombin challenge (Birukova et al., 2006). In contrast, physiologic CS levels (5% CS) facilitated Rac activation during the EC recovery phase leading to nearly complete EC monolayer recovery after 50 minutes of thrombin stimulation. Physiological CS preconditioning (5% CS, 24 hours) also enhanced EC paracellular gap resolution after step-wise increase to 18% CS (30 minutes) and thrombin challenge. More recent studies evaluated the potential protective effects of hepatocyte growth factor (HGF) on EC barrier dysfunction induced by CS and vascular endothelial growth factor (VEGF) (Birukova et al., 2008). HGF enhanced EC barrier function in a Rac-dependent manner and attenuated VEGF-induced EC permeability and paracellular gap formation in static cells. 5% CS further stimulated HGF-induced Rac signaling and enhanced cortical F-actin rim, whereas 18% CS promoted VEGF-induced Rho signaling, gap formation and EC permeability. Physiologic CS preconditioning combined HGF reduced the barrier-disruptive effects of VEGF via downregulation of the Rho pathway. These results suggest synergistic effects of HGF and physiologic CS in the Rac-mediated mechanisms of EC barrier protection and suggest an importance of physiologic mechanochemical environment in control of ALI/ARDS severity via regulation of lung endothelial permeability by a balance between different Rho family GTPases.

Concluding remarks

Small GTPases play central role in cytoskeletal remodeling, cell motility, control of endothelial permeability. Rac and Cdc42 mediated signaling is critical for recovery of monolayer integrity barrier restoration critical for resolution phase of ALI/ARDS. Although signaling by small GTPases and their effects on EC physiology are well recognized, the mechanisms of GTPase regulation by mechanical forces await further investigation. Understanding of the mechanisms of mechanotransduction and involvement of small GTPases in the regulation of endothelial permeability by combination of mechanical and chemical stimuli will identify key regulatory

molecules – targets for drug therapies and will lead to development of new therapeutic strategies in the treatment of VILI and ARDS. This area will remain to be an exciting field in the pulmonary research for years to come.

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Figure 1. Mechanochemical regulation of cytoskeletal remodeling and endothelial barrier by Rac and Rho GTPases

Endothelial cells in the lung ventilated at high tidal volume are exposed to pathological cyclic stretch (CS), which activates Rho-dependent signaling pathways and potentiates EC barrier-disruptive effects of inflammatory and edemagenic agents associated with VILI syndrome. In contrast, physiological CS, flow and barrier-protective agents (sphingosine 1-phosphate, hepatocyte growth factor, bioactive components of oxidized phospholipids) promote Rac-mediated signaling which contributes to EC barrier maintenance and restoration. Putative mechanosensors such as cell adhesion molecules PECAM and integrins, focal adhesion complexes, membrane-associated caveolae and stretch-activated ion channels convert mechanical signal to activation of biochemical cascades including signaling by small GTPases. Mechanisms of amplitude-dependent activation of Rac and Rho activities are not well understood, but may involve differential activation of Rac- and Rho-specific activators

(guanosine nucleotide exchange factors, GEFs) and inhibitors (GTPase activating proteins, GAPs). Precise spatial and temporal regulation of Rho and Rac activities may be mediated by cell adhesion- and caveolae-associated GEFs and GAPs and appears to be essential for the maintenance of EC barrier integrity in the pathological mechanochemical environment.