Microtubules-associated Rac regulation of endothelial barrier: a role of Asef in acute lung injury

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ABSTRACT

The endothelial barrier function regulated by the cytoskeletal reorganizations has been implicated in the pathogenesis of multiple lung diseases including asthma, sepsis, edema, and acute respiratory distress syndrome. The extensive studies have established that activation of small GTPase Rac is a key mechanism in endothelial barrier protection but the role of microtubules-associated Rac in the endothelial functions remains poorly understood. With the emerging evidences that microtubules disassembly also plays a critical role in actin cytoskeleton remodeling leading to endothelial permeability, the knowledge on microtubules-mediated regulation of endothelial barrier is imperative to better understand the etiology of lung injuries as well as to develop novel therapeutics against these disorders. In this regard, our recent studies have revealed some novel aspects of microtubules-mediated regulation of endothelial barrier functions and unraveled a putative role of Rac-specific guanine nucleotide exchange factor Asef in mediating the barrier protective effects of hepatocyte growth factor. In this review, we will discuss the role of this novel Rac activator Asef in endothelial barrier protection and its regulation by microtubules.

INTRODUCTION

Endothelial cells (EC) form a dynamic and semi-permeable barrier between the circulating blood and underlying tissues that precisely controls the passage of fluids, solutes, and immune cells. The increased EC permeability caused by pathogens, vasoactive and inflammatory agents leads to endothelial barrier dysfunction that is known to play a critical role in the etiology of several lung disorders including edema, sepsis, acute lung injury, and acute respiratory distress syndrome.[1–6] The EC barrier function is regulated by the cytoskeleton rearrangement and the loss of interendothelial junctions or formation of gaps results in EC hyperpermeability, ultimately causing endothelial barrier dysfunction as observed in many of the aforementioned lung diseases.[7–10] This process of cytoskeletal remodeling is primarily mediated by small GTPases Rac and Rho which play opposite roles, Rac being activated by barrier protective agents and Rho being the central pathway for many barrier disruptive agonists.[11–16] The activation of Rho-associated kinase is the major downstream effector of Rho that either directly phosphorylates myosin light chain or inactivates myosin light chain phosphatase by phosphorylation.[17 18] Both of these events lead to the actomyosin stress fiber formation and cell contraction, ultimately resulting in increased EC permeability.[19 20] Conversely, Rac antagonizes the barrier disruptive effects of Rho by stimulating peripheral actin polymerization and promoting the recovery of the disrupted cytoskeletal dynamics.[7 20] It is well established that the precisely controlled Rac and Rho activation regulates EC barrier function in physiological and pathological conditions. However, a role of microtubules (MT) in the modulation of this critical phenomenon that determines the fate of EC barrier remains to be elucidated. Here, we will focus on the role of Asef, a novel Rac activator, in mediating MT-induced cytoskeletal remodeling.

MT IN ENDOTHELIAL BARRIER REGULATION

MT are critical component of the cytoskeleton, carrying out the important functions of cell migration, shape and organelle transport.[21 22] MT are composed of α-tubulin and β-tubulin and their stability is controlled by acetylation and some stabilizing proteins such as stathmin and Tau.[23 24] Several studies have shown a direct link between the MT destabilization and EC barrier dysfunction induced by various agonists including thrombin and tumor necrosis factor-α.[25–29] However, role of MT in cytoskeletal reorganization leading to enhanced endothelial barrier functions remains to be explored. In this line of barrier protective roles of MT, our study had shown that atrial natriuretic peptide attenuates peptidoglycan-G-induced EC dysfunction by MT-mediated mechanism.[30] More importantly, our study revealed a novel Rac activator that associates with MT protein and mediates the barrier protective effects on EC which will be discussed in the next sections.

ASEF, A NOVEL MT-ASSOCIATED RAC ACTIVATOR IN ENDOTHELIAL BARRIER ENHANCEMENT

The molecular switch between active GTP-bound and inactive GDP-bound Rac is regulated by guanine nucleotide exchange factors (GEF). Among the Rac-specific GEFs, Asef has been implicated in the regulation of cytoskeleton remodeling in epithelial and neuronal cells.[31 32]

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Asef is composed of a Dbl homology domain with GEF activity, plekstrin homology domain that determines the subcellular localization, Src homology 3 autoinhibitory domain, and a binding region for tumor suppressor adenomatous polyposis coli (APC) protein.[33–35] We explored the role of Asef in the regulation of EC barrier function using hepatocyte growth factor (HGF) as a model of Rac-mediated EC barrier enhancement.[16] HGF increased Rac-1 specific nucleotide exchange activity of Asef and induced its membrane translocation.[36] A direct role of Asef in mediating HGF-induced EC barrier enhancement was confirmed with the results that overexpression of constitutively active Asef mimicked HGF's effects on cytoskeletal rearrangement and siRNA-mediated knock down or overexpression of dominant negative Asef attenuated these protective effects of HGF.[36] It appears that Asef is involved in local regulation of Rac with mainly targeting the activation of Rac at cell periphery which is consistent with Asef-controlled activation of actin regulatory protein cortactin and cytoskeletal remodeling at the cell periphery by HGF.[36]

On further delineating HGF-Asef signaling axis, our study showed that HGF activates Asef to induce endothelial barrier enhancement that is dependent on increased MT peripheral growth.[37] The role of MT in Asef-mediated HGF-induced EC barrier enhancement was further bolstered with the findings that Asef was associated with APC in MT-enriched fractions on HGF stimulation. Moreover, the translocation of APC–Asef complex to cell periphery was essential for full Rac activation and subsequent EC barrier enhancement. The MT-dependent regulation was specific to Asef since HGF also activated another Rac-specific GEF Tiam1 but its protective effects were not regulated by MT dynamics. Consistently, inhibition of MT peripheral dynamics reduced Asef translocation to cell periphery, resulting in attenuation of Rac and its effectors PAK1 and cortactin activation but had no any effects on Tiam1 trafficking.[37] These novel insights of Asef-mediated EC barrier enhancement prompted us to further investigate its role in EC barrier protection against various agonists.

Role of Asef in preventing acute lung injury: Asef for a safe lung?

Since we found that Asef acts in a cooperative manner with Tiam1 to mediate HGF-induced EC barrier enhancements, we next studied its sole role in preventing lung injury in vitro as well as in vivo. The increased levels of HGF in plasma and bronchoalveolar lavage (BAL) fluid during acute lung injury suggests that the elevated secretion of HGF serves as an endogenous cellular mechanism for lung recovery and HGF is known to have anti-inflammatory effects.[38 39] To investigate if Asef mediates HGF-induced endothelial barrier function and lung recovery, we employed lipopolysaccharide (LPS) as an inflammatory agonist in cultured EC and mice. Our results demonstrated that HGF offered protection against LPS-induced EC hyperpermeability by inhibiting the nuclear factor kappa-light-chain-enhancer of activated B cells pathway and repressing the expression of EC surface adhesion molecules and inflammatory cytokines.[40] More importantly, Asef played a critical role in mediating the protective effects of HGF against LPS-induced EC barrier dysfunction since barrier protective and anti-inflammatory effects of HGF were attenuated by the siRNA-mediated knock down of Asef. The importance of Asef in mediating the protective effects of HGF established by these cell culture studies were reproduced in rodent model of acute lung injury where HGF-induced protection against lung inflammation and vascular leak caused by LPS was reduced in Asef knockout mice.[40]

The role of Asef in mediating the HGF-induced protection against EC barrier dysfunction was not limited to LPS. The depletion of Asef with siRNA impaired the protective effects of HGF against thrombin-induced EC permeability and formation of actin stress fibers and intracellular gaps.[36] Here, Asef mediated the protective effects of HGF by inhibiting thrombin-induced Rho GTPase activation and subsequent Rho-dependent cytoskeleton rearrangement leading to EC barrier dysfunction. We also tested the role of Asef in vivo in two-hit model of acute lung injury with excessive mechanical ventilation followed by treatment with thrombin-related signaling peptide TRAP6. HGF successfully attenuated lung inflammation and vascular leak induced by two-hit model lung injury in wild type mice but these protective effects of HGF were abolished in Asef knockout mice.[36] These observations strongly suggest that Asef is indispensable for mediating the protective effects of HGF against both LPS and thrombin-induced lung injury models. In addition, Asef-mediated barrier protection by HGF was in accordance with the most conventional pathway employed by several barrier protective agents where they activate Rac to suppress agonists-activated Rho and inhibit Rho-dependent cytoskeleton remodeling.

Mechanism of Asef-mediated endothelial barrier protection

Once our studies established that on HGF stimulation, Asef gets translocated to the cell periphery by forming a complex with MT-associated protein APC and thereby plays an important role in peripheral Rac activation and cytoskeleton remodeling, we next examined whether Asef interacts with other Rac effectors in this process. A multifunctional adaptor protein IQGAP1 is known to control MT and actin cytoskeletal dynamics by interacting with Rac and it also directly interacts with APC and MT-associated plus end tracking protein CLIP-170.[41–44] Based on these findings, we investigated whether Asef interacts with IQGAP1 during HGF-induced EC barrier enhancement. Our results showed that HGF indeed stimulates the formation of a functional protein complex of Asef and IQGAP1 at the cell cortical area.[45] Asef was essential for HGF-induced Rac activation and its association with IQGAP1. Furthermore, the translocation of IQGAP1 at the cell cortical area and its interaction with cytoskeletal regulators coractin and Arp3 was also dependent on Asef.[45] The nucleotide exchange activity of Asef was necessary for its interaction with IQGAP1 and to form a protein complex at the cell periphery. For IQGAP1, its C-terminal domain was critical for the interaction with Asef. These cumulative evidences strongly indicate that HGF-induced/Rac-mediated remodeling of cortical actin dynamics and EC barrier enhancement is facilitated by the stimulation of Asef and its interaction with IQGAP1 (figure 1). This novel phenomenon might serve as a feedback mechanism of HGF-induced

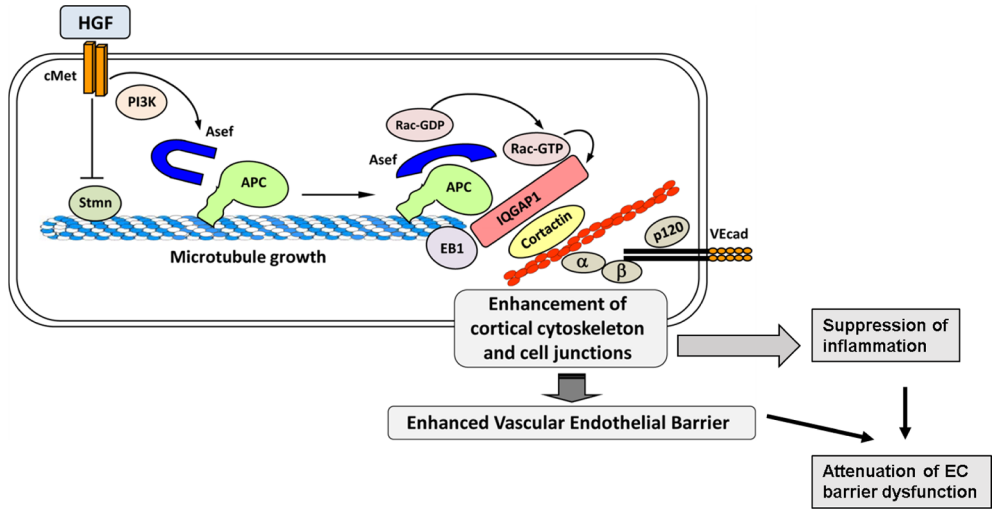

Figure 1 Proposed mechanism of Asef-mediated endothelial cells (EC) barrier enhancement via microtubules (MT)-associated Rac-dependent manner. HGF activates Asef and stimulates its association with MT protein APC leading to the peripheral translocation of the Asef–adenomatous polyposis coli (APC) protein complex. Asef forms a protein complex with IQGAP1 at the cell periphery by binding to its C-terminal domain and induces the local activation of Rac to promote EC barrier enhancement. The remodeling of cytoskeletal dynamics by Asef–IQGAP interaction also suppresses vascular inflammation and attenuates EC barrier dysfunction.

EC barrier enhancement that controls the level of HGF-induced Rac activation and maintains cortical cytoskeletal rearrangement accordingly.[45] Moreover, the role of IQGAP as an important linker between MT and Rac-mediated EC barrier enhancement was further confirmed in our other study where HGF stimulation induced Rac-dependent IQGAP1 association with end-binding (EB1) protein in MT.[46]

CONCLUSION

The growing evidences suggest that the dynamic interaction between MT and actin cytoskeleton plays a critical role in cytoskeletal remodeling that is essential for EC barrier enhancement. Rac activation is the central pathway to mediate the barrier protective effects and our studies establish that Asef is a novel Rac activator in pulmonary EC which is indispensable for HGF-induced protective effects against multiple agonists both in vitro and in vivo. Asef-induced peripheral activation of Rac and rearrangement of cortical cytoskeletal dynamics occurs only after its association with MT protein APC, highlighting an important role of MT in Asef-mediated local regulation of Rac. Furthermore, Asef also interacts with IQGAP1, a major Rac effector in modulating cortical actin dynamics, and this interaction is essential for inducing cortical cytoskeletal rearrangement and EC barrier protection. Moreover, the ability of IQGAP1 to interact with EB1 to regulate EC barrier function warrants a further investigation whether it also associates with other MT proteins such as CLIP-170, CLASP2 to modulate cytoskeletal remodeling in pulmonary EC. To sum up, along with the MT stabilization, MT-associated regulation of EC barrier function appears to play an important role in mediating the protective effects of various agents and future studies to unravel MT-related signaling in the regulation of pulmonary endothelium could lead to novel therapeutics against a wide range of lung disorders.

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Competing interests None declared.

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