Poster J-15

The Role of LIMK1 in Endothelial Barrier Function

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Short Abstract: Microtubule destabilization promotes the formation of actin stress fibers and enhances the contractility of cells. Our findings indicate that LIM kinase 1, LIMK1 can coordinate microtubules and actin cytoskeleton. We showed that the loss of LIMK1 protein leads to less permeable pulmonary blood vessels. Therefore, LIMK1 might be a target for therapeutic drug development for acute lung injury and lung edema.

Long Abstract:
The Role of LIMK1 in Endothelial Barrier Function Matvey Gorovoy, Tatyana Voyno-Yasenetskaya Department of Pharmacology, College of Medicine, University of Illinois at Chicago Microtubule (MT) destabilization promotes the formation of actin stress fibers and enhances the contractility of cells; however, the mechanism involved in the coordinated regulation of MTs and the actin cytoskeleton is poorly understood. LIM kinase 1 (LIMK1) regulates actin polymerization by phosphorylating the actin depolymerization factor, cofilin. Here we report that LIMK1 is also involved in the MT destabilization. In endothelial cells endogenous LIMK1 co-localizes with MTs and forms a complex with tubulin via the PDZ domain. MT destabilization induced by thrombin or nocodazole resulted in a decrease of LIMK1 colocalization with MTs. Overexpression of wild type LIMK1 resulted in MT destabilization, whereas the kinase-dead mutant of LIMK1 (KD) did not affect MT stability. Importantly, down-regulation of endogenous LIMK1 by small interference RNA resulted in abrogation of the thrombin-induced MTs destabilization and the inhibition of thrombin-induced actin polymerization. Expression of Rho kinase 2, which phosphorylates and activates LIMK1, dramatically decreases the interaction of LIMK1 with tubulin but increases its interaction with actin. Interestingly, expression of KD-LIMK1 or small interference RNA-LIMK1 prevents thrombin-induced microtubule destabilization and F-actin formation, suggesting that LIMK1 activity is required for thrombin-induced modulation of microtubule destabilization and actin polymerization. We examined pulmonary vascular permeability in LIMK1 knockout mice. We found that endothelial permeability in the lungs of LIMK1 -/- mice was lower than that of wild type mice. Perfusion of the lungs of wild type mice with PAR1 peptide showed significant increase of endothelial permeability. Notably, the endothelial permeability of the lungs of LIMK1 -/- mice after PAR1 peptide perfusion was significantly lower than that of wild type. Acute lung injury (ALI) is a syndrome of acute respiratory failure that results from acute pulmonary edema and inflammation. Using lipopolysaccharide (LPS) injection as a model of ALI, we have shown that LIMK1 (-/-) mice did not develop lung edema and displayed significantly reduced mortality as compared to wild type mice. Our findings indicate that LIMK1 may coordinate microtubules and actin cytoskeleton. We suggest that the loss of LIMK1 protein leads to less permeable pulmonary blood vessels. Therefore, LIMK1 might be a target for therapeutic drug development for acute lung injury, lung edema, and diabetic retinopathy.