INTRODUCTION. Crosstalk between microtubules and the actin cytoskeleton is essential for the regulation of many cellular functions such as migration, locomotion, cytokinesis, and cell polarity (reviewed in (1). However, the mechanism of agonist-dependent microtubule disassembly is not yet understood. We have shown here that serine/threonine protein kinase LIMK1 induces microtubule depolymerization and actin polymerization and propose that LIMK1 may serve as a molecular switch that regulates both microtubule disassembly and formation of actin stress fibers, thereby providing the molecular mechanism that explains how microtubule disassembly promotes the formation of actin stress fibers.

METHOD. Role of LIMK1 in microtubule disassembly and actin polymerization in human endothelial cells was evaluated using quantitative confocal microscopy, immunoprecipitation assays, in vitro protein binding assays, and in vitro kinase assays.

RESULTS. The serine/threonine protein kinase LIMK1 is a key regulator of the actin cytoskeleton. Here we report that LIMK1 is also involved in the depolymerization of microtubules. In endothelial cells, endogenous LIMK1 co-localizes and forms a complex with microtubules via its PDZ domain. Microtubule depolymerization induced by thrombin or nocodazole decreased LIMK1 interaction with tubulin and resulted in LIMK1 translocation to sites of increased actin dynamics. Expression of ROCK2, which phosphorylates and activates LIMK1, dramatically decreases the interaction of LIMK1 with tubulin but increases its interaction with actin. Importantly, overexpression of wild type LIMK1, but not its kinase-dead mutant, resulted in microtubule depolymerization. In endothelial cells LIMK1 translocates to and co-localizes with F-actin only after stimulation with thrombin. Interestingly, kinase-dead mutant of LIMK1 prevented microtubule depolymerization and actin stress fiber formation induced by thrombin suggesting that LIMK1 activity was required for the thrombin-induced modulation of microtubule depolymerization and actin polymerization. Our findings indicate that LIMK1 may coordinate microtubules and actin cytoskeleton.

DISCUSSION. LIMK1 and crosstalk between microtubules and actin cytoskeleton

The role of LIMK1 in regulation of actin dynamics is well established. LIMK1 regulates actin dynamics via phosphorylation and inactivation of cofilin (2). In agreement with previous findings, our data also demonstrate that LIMK1 induces actin stress fiber formation. In addition, we have shown that kinase-dead mutant of LIMK1 attenuated actin stress fiber formation induced by thrombin and that stabilization of microtubules with taxol, prevented both translocation of LIMK1 to F-actin and actin polymerization.

The Rho family of small GTPases was shown to participate in the regulation of both microtubules and actin (3). Importantly, microtubule disassembly was shown to induce Rho activation (4). Microtubule disassembly releases the microtubule-bound Rho guanine nucleotide exchange factor (GEF), GEF-H1 to activate RhoA (5). However, the mechanism of agonist-dependent microtubule disassembly is not yet understood. We have shown here that LIMK1 induces microtubule depolymerization and actin
polymerization and propose that LIMK1 may serve as a molecular switch that regulates both microtubule disassembly and formation of actin stress fibers, thereby providing the molecular mechanism that explains how microtubule disassembly promotes the formation of actin stress fibers (Figure 1).

Ligand-induced activation of Rho-ROCK pathway activates LIMK1, which in turn causes microtubule depolymerization and release of LIMK1 to the cytoplasm. Consequently, activated LIMK1 associates with actin, thereby inducing its polymerization via cofilin phosphorylation.

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