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LIM kinase-mediated regulation of cytoskeletal dynamics in mouse submandibular salivary gland branching morphogenesis

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Branching morphogenesis in the mouse submandibular gland is a complex and dynamic process involving several structural changes in the epithelium, including dynamic regulation of cytoskeletal stability, cell migration, and cell-cell adhesions to control formation of clefts, or indentations in the epithelial buds. In this study, we report that LIM kinase (LIMK), a dual-specificity serine/threonine kinase that is regulated by Cdc42 and Rac GTPase in early embryonic salivary glands, is required for branching morphogenesis and is a master regulator of these structural changes. We utilized LIMKI/II siRNA and pharmacological LIMK inhibitors – BMS-3 and BMS-5 - to show that LIMK I/II functions in a myosin-independent pathway to increase cofilin phosphorylation thereby inactivating it, and hence stabilize F-actin filaments in embryonic day 13 salivary glands. LIMK I/II inhibition was previously reported to stabilize microtubules through interaction with p25/tubulin polymerization promoting protein (TPPP). Accordingly, use of TPPP siRNA caused a decrease in branching and a similar increase in stabilized microtubules. Interestingly, 24 hours of incubation with LIMK I/II siRNAs caused destabilization of clefts. Cleft destabilization was also observed in mesenchyme-free epithelial cultures treated with a LIMK inhibitor (BMS-3) that negatively impacts both the actin- and microtubule-dependent effects of LIMK, whereas an inhibitor that only affects only F-actin organization (BMS-5) prevented initiation of new clefts, as detected by time-lapse microscopy. Whereas BMS-3 drastically reduced cell-cell adhesions and cell proliferation, BMS-5 did not affect cell-cell adhesions or cell proliferation. We utilized computational analysis to determine that BMS-5 significantly reduced cell area relative to control while BMS-3 did not. Using time-lapse confocal microscopy to track movement of nuclei in BMS-5 inhibitor-treated, mesenchyme-free epithelial rudiments, we found that LIMK also inhibited epithelial migration in an actin-dependent manner. We propose a mechanism whereby LIMK regulates actin polymerization and microtubule stability to control epithelial cell-cell adhesion and cell migration to affect both cleft formation and cleft stability in developing salivary glands. Supported by NIH/NIDCR RO1 DE019244.