Hedgehog Signaling Regulates Apical Actin Morphology

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Stereocilia are highly patterned actin based cell protrusions found on the apical surface of auditory hair cells. They are formed mainly from bundled filamentous actin and its associated actin cross-linking proteins. Interestingly, stereocilia develop around another cell appendage, the microtubule based kinocilium, which is the primary cilium for a hair cell. Primary cilia are found on most somatic cells and play a significant role in the regulation and proper transduction of the Hedgehog (Hh) pathway. In the current study, we are testing the hypothesis that Hh pathway activity can alter actin bundling and elongation. In support of this idea, ectopic activation or repression of Hh signaling changed the morphology of stereocilia *in vivo*. To further test our hypothesis, we used a CL4 porcine kidney epithelial cell culture system stably expressing the actin crosslinking protein ESPN fused to green fluorescent protein. These cells serve as an *in vitro* model of apical actin protrusions similar to mature stereocilia *in vivo*. We manipulated Hh signaling in these cells using both a genetic and a pharmacological approach. In the pharmacological approach, CL4 cells were treated with the hedgehog agonist (Purmophamine) and antagonist (Cyclopamine), at varying concentrations for 48 hours. Genetically, the Hh pathway was ectopically activated by overexpressing the transcription factor Gli1, Gli2, Gli3, and SmoA1 repressed by expressing Gli3R. Immunofluorescent (IF) and scanning electron microscopy (SEM) revealed that CL4 cells dramatically altered the apical actin structures under these conditions. In particular, activating Gli transcription decreased apical actin-based structures while antagonizing activity resulted in more actin-based protrusions. This data strongly supports the hypothesis that the Hh signaling pathway can regulate the actin cytoskeleton.

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