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Integrin-linked kinase in ciliary Hedgehog signaling

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Hedgehog is a conserved developmental pathway governing embryo patterning, tissue morphogenesis and differentiation. Vertebrate Hh signaling is coordinated in the primary cilium, a MT (MT)-based structure derived from the basal body (centriole) that projects singly from the surface of most cells. Defects in cilia and Hedgehog signaling cause developmental disorders affecting a range of organ systems, and recent years have witnessed an increasing appreciation of the role of cilia and dysregulated Hh signaling in promoting cancer.[1]

The integrin-linked kinase, ILK, was discovered and is best understood as a focal adhesion protein, playing a conserved role in assembling multi-protein signaling complexes at integrin attachment points that regulate cell proliferation, differentiation, migration and survival.[2] However, additional functions of ILK localize via distinct interactions with non-focal adhesion partners. We have now discovered that ILK promotes Hedgehog-dependent development of the cerebellum, a mammalian hindbrain structure responsible for motor coordination, and moreover have identified novel ciliary protein interactions suggesting direct involvement of ILK in the Hh pathway, through physical engagement with its signaling machinery.[3]

Hh signaling occurs via activation of the GPCR-like protein Smoothened (Smo), which is translocated in a ligand-dependent manner from endomembranes into the primary cilium, where it in turn activates Gli transcription factors to generate mature signal.[4] Hh signaling is deficient in ciliated cells in which ILK has been either knocked down by siRNA, or had its kinase activity inhibited by a selective small molecule. Although ciliary integrity is required for Hh signaling, defective Hh signaling in ILK-inhibited cells is the result of markedly impaired Smo translocation.[3] This result suggests that signaling, rather than regulation of cilia assembly or maintenance, is the primary function of ILK in this organelle. Indeed, quantitation of cilia number and length in ILK-depleted cells indicates no obvious effect on MT dynamics controlling cilia structure.[3] This is somewhat surprising, in light of previous results demonstrating ILK interactions with MT-associated proteins to organize the mitotic spindle.[5] Thus, removed from focal adhesions, diverse functions of ILK are specified through interactions in discrete MT-associated complexes, for example with the centrosomal proteins RuvB and chTOG during organization of mitotic spindles.[5]

ILK ciliary targetting does not require binding to its focal adhesion partners, Parvins or PINCH. We have localized interactions of ILK with Hh ciliary proteins using bimolecular fluorescence complementation (Split Venus), a technique requiring proximity of about 30 nm between protein partners to generate fluorescent interaction signals. Selective association of ILK at the basal body with β-arrestin (Barr), a GPCR-associated adaptor that mediates Smo ciliary translocation,[6] is likely to be important for localization and ultimately, interaction of ILK with Smo in the cilium.[3] Although very close, whether ILK interactions with Barr or Smo are direct is not definitively established, nor is it known how ILK gets into the cilium, as anterograde transport is tightly regulated. Kinesin II motors accumulate at the ciliary base and comprise the intraflagellar transport (IFT) machinery responsible for anterograde movement of protein cargo,[1] thus IFT seems a likely vehicle for delivery of ILK/Barr complexes across the transition zone (TZ, **Fig. 1**) into the cilium. The presence of overlapping Barr complexes with ILK and the Kinesin II subunit, Kif3a, at the TZ of unstimulated cells (our unpublished data) suggests that Hh stimulates transport of tripartite complexes into the cilium (**Fig. 1**). Critically important, then, is the question of how these complexes engage Smo to promote signaling. Hh signaling is sensitive to a small molecule ILK kinase inhibitor, therefore translocation may involve Hh-stimulated phosphorylation of Barr or Kif3a by ILK. Unfortunately, resolution of this question is complicated experimentally by the fact that Kif3a is required for elaboration of cilia. In addition to identifying the mechanism underlying its targetting to cilia, the dynamics of ILK ciliary transport need to be defined. ILK residency along the length of the cilia of unstimulated cells suggests a basal rate of anterograde/retrograde cycling, which increases in a kinase activity-dependent manner under the influence of Hh ligand to rapidly deliver Smo to the ciliary tip (**Fig. 1**). Distinct IFT proteins regulate retrograde ciliary transport, thus this model predicts that mutation of a relevant retrograde IFT protein would block ILK egress and increase its accumulation in the cilium. Again, experimental difficulties in directly addressing this question

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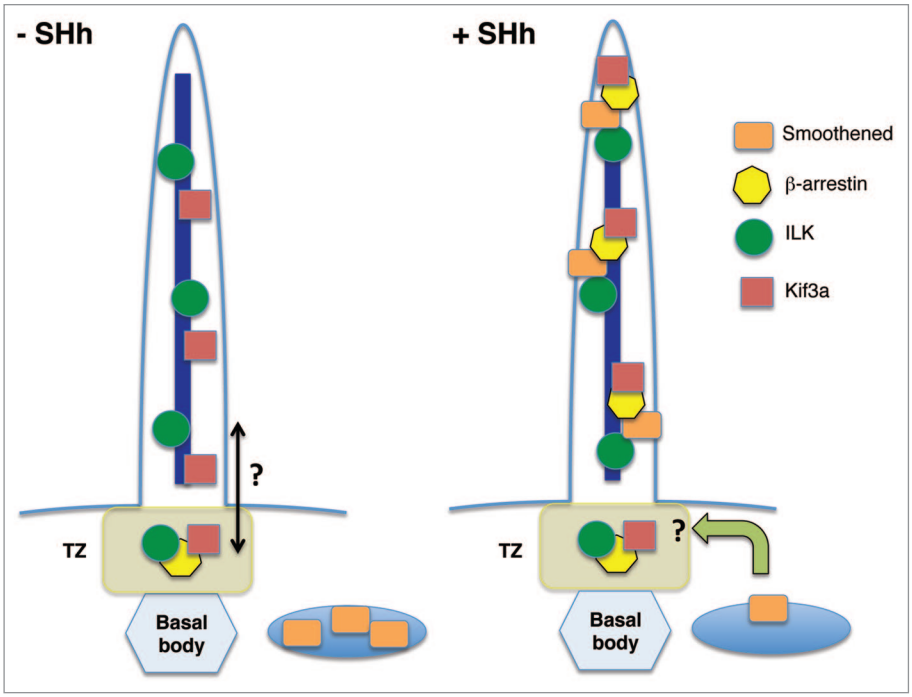

Figure 1. In unstimulated cells Smo is sequestered in cytoplasmic vesicles, and ILK and β-arrestin form a complex with Kif3a in the TZ. Double-headed arrow indicates low basal rate of cycling of ILK between the TZ and cilium (left). SHh stimulates Smo translocation from endomembranes by a poorly understood mechanism involving interaction with β-arr and ILK (right).

relate to the structural role of IFT in cilia. Nonetheless, dynamic photobleaching experiments and identification of a potential ILK-cognate retrograde IFT protein are priorities in defining the molecular details of ILK ciliary signaling.

As many common cancers are caused by or exhibit aberrant Hh signaling, including basal cell, pancreatic and breast carcinomas, inhibitors of Hh signaling are currently of great interest as cancer therapies.[1] Medulloblastoma (MB) the most common solid malignancy of childhood, and is an aggressive cancer caused by activated Hh in granule cell precursors (GCPs) of the developing cerebellum. Mouse models targetting Hh activation to GCPs provide a rigorous, clinically relevant platform on which to study the role of ILK in Hh-driven MB.[7] We predict that deletion of ILK from GCPs will significantly delay the incidence or progression of Hh-activated MB in these mice. ILK is a druggable molecule,[8] thus an exciting possibility is that pre-clinical results from our mouse models will present a strong case for further evaluation of ILK as a therapeutic target in cilia- and Smo-dependent Hh cancers.

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