Cancer Cell
Previews

Tail Wags Dog: Primary Cilia and Tumorigenesis

Monique T. Barakat[1] and Matthew P. Scott[2,*]
[1]Medical Scientist Training Program, Program in Neurosciences
[2]Departments of Developmental Biology, Genetics, and Bioengineering
Howard Hughes Medical Institute, Clark Center West W252, 318 Campus Drive, Stanford University School of Medicine, Stanford, CA 94305-5439, USA
*Correspondence: mscott@stanford.edu
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Aberrant activation of the Hedgehog (Hh) signaling pathway contributes to many forms of cancer. Primary cilia are Hh signal transduction centers. Two papers in a recent issue of *Nature Medicine* (Han et al., 2009; Wong et al., 2009) show that mutating cilia can increase or reduce the rates of tumorigenesis depending on how the Hh pathway is disrupted.

In a victory for a small and once neglected organelle, two papers recently published in *Nature Medicine* now demonstrate that brain and skin tumors dependent on Hedgehog (Hh) signaling are regulated by primary cilia (Han et al., 2009; Wong et al., 2009). Erudite cell biologists have known for a long while that most cell types in our bodies have these singular immotile appendages (Berbari et al., 2009). Genetic damage to primary cilia results in a spectrum of problems classified as ciliopathies. The diseases can have a range of effects, including blindness and improper brain development (Sharma et al., 2008). Primary cilia are important for proper Hh signaling (Figure 1), and at least some ciliopathy-associated problems can now be attributed to disrupted Hh signal transduction.

Motile cilia have familiar active physiologic roles, such as clearing air passages and moving cerebrospinal fluid through ventricles in the brain. In contrast, primary cilia are moved rather than movers, transducing signals in response to fluid flow in kidneys or in response to mechanical bone deformation. Cilia consist of a plasma membrane sheath containing a microtubule-based axoneme with nine peripheral microtubule doublets and no central pair of microtubules. The axoneme extends from the basal body, which arises from a centriole taking time off from cell division. When cells reenter the cell cycle, the centriole must return to its role in cell division, and primary cilia are disassembled. These intermittently present cell-surface organelles contain a rich collection of proteins, as well as mysteries.

Kif3a and *Ift88* encode cilium components. *Kif3a* encodes a kinesin that is employed for transport along the microtubule axoneme from the base of cilia toward their tips. Because protein synthesis does not occur in cilia, this anterograde transport is crucial in formation and maintenance of the organelle. Mice lacking Kif3a function lack cilia, die early in embryonic development, and exhibit deficient left-right patterning (Marszalek et al., 1999). *Ift88* encodes a component of the intraflagellar transport machinery that is required for cilium formation. Ift88 protein is present in basal bodies and cilia in nondividing cells, and in centrosomes during cell division (Robert et al., 2007). Mice lacking Ift88 function die in midgestation. Their defects include left-right asymmetry, cilium malformation, and neural-tube abnormalities (Murcia et al., 2000).

Key features of Hh signaling were initially worked out in *Drosophila*, an organism in which Hh-transducing cells lack primary cilia. Appreciation of the involvement of cilia in vertebrate Hh signaling has provoked new questions about cell biology mechanisms. When Hh pathway restraint systems are damaged, cancer can arise in tissues where Hh target genes normally stimulate cell division. The Hh pathway was first connected to cancer when Gorlin syndrome was found to be due to loss of Ptc receptor function. Loss of Ptc function allows Smo activity to go unchecked, thus leading to excessive activation of Hh target genes that promote cell division. The consequences, in Gorlin syndrome, include basal cell carcinoma (BCC) of the skin and medulloblastoma (MB) of the cerebellum, as well as skeletal and other defects.

The two new papers exploit another route to tumorigenesis—constitutively active Smo protein encoded by the Smo^{M2} allele that is capable of causing cancer despite normal Ptc protein activity. In the first paper, Wong and colleagues showed that human and mouse skin tumor cells have cilia, at least some of the time, as does normal mouse skin. The authors generated conditional Smo^{M2} mice that enable tamoxifen-inducible, skin-restricted production of the Smo^{M2} protein. After tamoxifen treatment at age 30 days, hyperactive Smo^{M2} was produced. Within 5 wks, mice began to exhibit epidermal hyperplasia and a premalignant lesion, and substantial penetrating growths into the dermis appeared by 10–20 wks. When Cre was used to produce Smo^{M2} and at the same time inactivate a floxed allele of *Kif3a* in skin cells, BCC tumorigenesis was blocked. The skin and tumors of these mice had few cilia, as expected.

The Gli2 transcription factor acts downstream of Smo in the skin, so the authors further investigated the role of Kif3a in tumorigenesis, using a mouse model employing inducible constitutively active Gli2 with a mutation in the N-terminal repressor domain of the protein (Gli2ΔN). Gli2ΔN, behaving as a constitutively active protein, raises the levels of endogenous Gli2. Here came the big surprise: tumorigenesis was *accelerated* in the absence of cilia. Evidently, cilia can normally restrain the activity of this abnormal form of Gli2. The use of Gli2ΔN makes the implications for typical tumorigenesis unclear, but the results could imply that sequestration of normal Gli2 in cilia limits its ability to go off and cause trouble in the nucleus. Cilia are also needed to generate a repressing form of Gli3 (GliR).

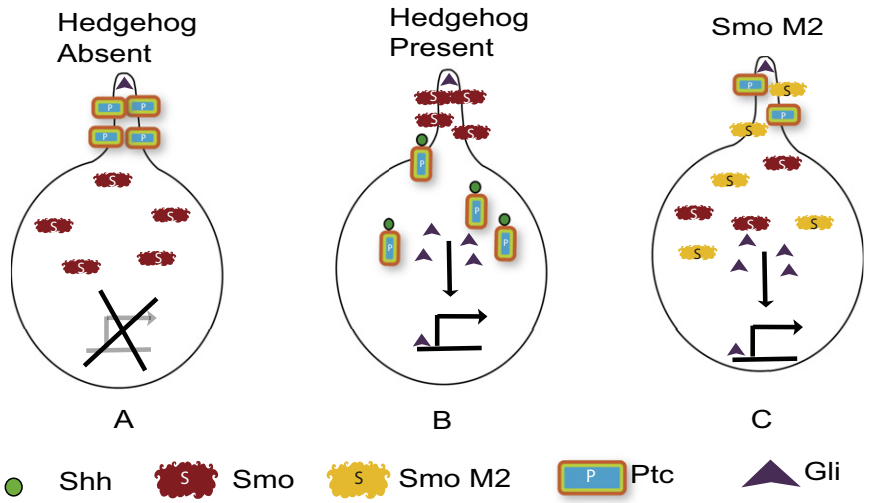

Figure 1. Hedgehog Signal Transduction in Development and Cancer
In the Hh pathway, binding of Hh ligand to the Patched (Ptc) 12-transmembrane domain receptor unleashes the 7-transmembrane domain protein Smoothened (Smo) to direct the formation of activating transcription factors, Gli proteins, that trigger target gene expression. Intracellular transport events occur during Hh transduction. In the absence of Hh ligand (A), Ptc is in cilia and Smo is largely outside cilia. Gli transcription factors are, remarkably, present in cilia. Processing of Gli proteins can give rise to repressor forms that go to the nucleus and repress target genes. When Hh ligand arrives at the primary cilium (B), it binds Ptc, and together they leave the cilia. The absence of Ptc from cilia, either upon Hh binding or when *ptc* is mutated, allows Smo to move into cilia, where it is evidently capable of triggering processing and transport of activating Gli transcription factor forms to the nucleus. Constitutive Smo activity in Smo^{M2} mutant cells (C) leads to activation of Gli transcription factors and target gene expression, even in the absence of Hh ligand.

Thus, Gli3R may restrain Gli2ΔN in ciliated cells. When cilia are not formed, Gli3R cannot be produced and Gli2ΔN has added destructive potency. How Gli2ΔN interacts with normal Gli proteins is unclear.

The second paper applies a similar strategy to the analysis of MB, a less frequent but more deadly tumor often seen in Gorlin syndrome patients. The paper reports the presence of cilia in human MBs. To test the roles of cilia in MB, Han and colleagues expressed the constitutively active Smo^{M2} allele or the *Gli2ΔN* construct, using human glial fibrillary acidic protein (hGFAP) promoter-driven Cre, which directs expression of Cre in granule neuron precursors (GNPs) of the cerebellum, among other primitive neural cells. By postnatal day 10, prior to complete development of the cerebellum, mice with hGFAP-driven Smo^{M2} expression expanded their GNP population and formed tumors. Normally, GNPs proliferate under the influence of Sonic hedgehog signal emanating from Purkinje neurons, and GNPs are thought to be the cell type of origin for MBs.

In the new work, removal of Kif3a and Ift88, and the consequent loss of cilia, blocked normal proliferation of GNPs and tumorigenesis. In contrast to hGFAP-driven Smo^{M2} expression, activation of the Hh pathway with the use of hGFAP-driven Gli2ΔN expression failed to induce MBs. Gli2ΔN transgenic mice developed brain tumors only after removal of Kif3a and primary cilia; just as in BCCs, Gli2ΔN-triggered tumorigenesis is enhanced. Brain tumors in these hGFAP-driven *Gli2ΔN* and Smo^{M2} mice differed from each other and from Gorlin syndrome tumors arising from *Patched* haploinsufficiency. Smo^{M2} mutant mice have tumors that encase the entire cerebellum as a result of rapid malignant transformation of all GNPs, as compared to Gorlin syndrome tumors, in which a discrete tumor forms from a small population of GNPs. A subset of Gli2ΔN tumors (termed type 1 tumors) resembles MB. hGFAP-Gli2ΔN mice also develop a somewhat mysterious second type of central nervous system tumor, which can be located in the cerebellum, brainstem, or cerebrum and is histologically distinct from MB. The significance of type 2 tumors, and their relevance to Hh signaling and human disease, remains unclear.

Thus, the character of skin and neuron precursor cells can be dramatically transformed by tiny tails that exert a disproportionately large influence on cell fates. Developmental signaling pathways, including Hedgehog, Wnt, TGFβ, and Notch, have been implicated in oncogenesis, so their constituent proteins have become candidates for new cancer drug targets. Recent papers have demonstrated the utility of Smo antagonists in Hh-driven human tumors, including BCC and MB (Rudin et al., 2009; Von Hoff et al., 2009; Yauch et al., 2009). The two recent *Nature Medicine* papers highlight the intriguing potential of primary cilia components as alternative targets for antitumor drugs, but they underscore the challenges of manipulating an organelle that has positive and negative effects on pathway activity and tumorigenicity.

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