The Primary Cilium in Cell Signaling and Cancer

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Abstract

The primary cilium is a microtubule-based antenna-like structure that emanates from the surface of virtually all cells in the mammalian body. It is anchored to the cell by the basal body, which develops from the mother centriole of the centrosome in a manner that is coordinately regulated with the cell cycle. The primary cilium is a sensory organelle that receives both mechanical and chemical signals from other cells and the environment, and transmits these signals to the nucleus to elicit a cellular response. Recent studies revealed that multiple components of the Sonic hedgehog and platelet-derived growth factor receptor-α signal transduction pathways localize to the primary cilium, and that loss of the cilium blocks ligand-induced signaling by both pathways. In light of the major role that these pathways play in numerous types of cancer, we anticipate that the emerging discoveries being made about the function of the primary cilium in signaling pathways that are critical for embryonic development and tissue homeostasis in adults will also provide novel insights into the molecular mechanisms of carcinogenesis. (Cancer Res 2006; 66(13): 6463-7)

Introduction

Inappropriate activation of signaling molecules and receptors that are important for controlling cellular migration, proliferation, differentiation, and apoptosis could lead to the development of many different types of cancer. Examples of two such signal transduction pathways that have essential roles in embryonic development and tissue homeostasis in adults, and when over-activated, cause cancer in numerous tissues, are the Sonic hedge-hog (Shh) and platelet-derived growth factor receptor-α (PDGFRα) pathways. Loss of Shh signaling causes widespread defects in embryonic development, including dorsal-ventral patterning defects, randomized left-right axis specification, and reduced limb length and digit number (1), whereas unregulated activation of the pathway is associated with oncogenesis in the skin, brain, lung, pancreas, and prostate (2). Similarly, loss of PDGFRα signaling causes mid-gestation embryonic lethality with defects in many organ systems (3), and unregulated activation of the pathway is associated with numerous tumor types, notably gastrointestinal stromal tumors (4). Because the Shh and PDGFRα signal trans-duction pathways are altered in numerous cancers, it is intriguing that recent discoveries revealed that the primary cilium is essential for ligand-induced activity of both pathways. Thus, we anticipate that the increasing attention now being paid to cilia structure and function will reveal new insights into the pathogenesis of many types of cancer, and that some cilia proteins may represent new targets for cancer therapeutics. Here, we provide a brief overview of the primary cilium and its role as a mediator of Shh and PDGFRα signaling.

## Evolutionary Conservation of the Eukaryotic Primary Cilium

Cilia and flagella are microtubule-based organelles projecting from the surface of cells and are conserved in eukaryotes ranging from the single-celled green algae, *Chlamydomonas*, to humans. Cilia can be grouped into three general categories. Most people are familiar with the whip-like action of motile cilia, which usually occur in groups on cells and have an axoneme, or core, composed of nine pairs of outer microtubules surrounding a single pair of inner microtubules (9 + 2 arrangement). Examples of motile cilia in vertebrates are those on the epithelial lining of the lung that move mucus, on ependymal cells lining brain ventricles that circulate cerebrospinal fluid, and on cells lining the oviducts and testes that move germ cells. Fewer people are familiar with nodal cilia and primary cilia, which both have an axoneme with a 9 + 0 microtubule arrangement. Nodal cilia occur singly on cells of the embryonic node in vertebrates. They exhibit a rotational move-ment involved in the generation of leftward extraembryonic fluid flow and the establishment of morphogen gradients essential for left-right axis specification (5). Primary cilia are immotile and occur singly on most epithelial and stromal cells throughout the mammalian body, with the exception being differentiated cells of myeloid or lymphoid origin (6, 7).[3] Primary cilia are very small (~0.2 μm in diameter and ~5 μm in length) and are usually observed with an electron microscope (Fig. 1A), or more routinely with an epifluorescence microscope following immunofluorescent labeling with α-tubulin antibodies (6, 7). The primary cilium was once thought to be an evolutionary vestige in vertebrates. However, recent studies revealed that the primary cilium is an essential sensory organelle in many tissues, and genetic mutations that disrupt the function of primary cilia result in a broad spectrum of disorders, including cystic kidneys, hepatic and pancreatic abnor-malities, skeletal malformations, obesity, and severe developmental defects (8–11).

The plasma membrane of the cilium is continuous with the remainder of the cell; however, protein entry into the cilium is tightly controlled and does not occur by simple diffusion along the membrane. Because the cilium is devoid of ribosomes, pro-teins targeted to the cilium must be transported from their site of synthesis in the cytosol into the cilium where they are integrated

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[3] http://members.global2000.net/bowser/cilialist.html (Where are primary cilia found?).
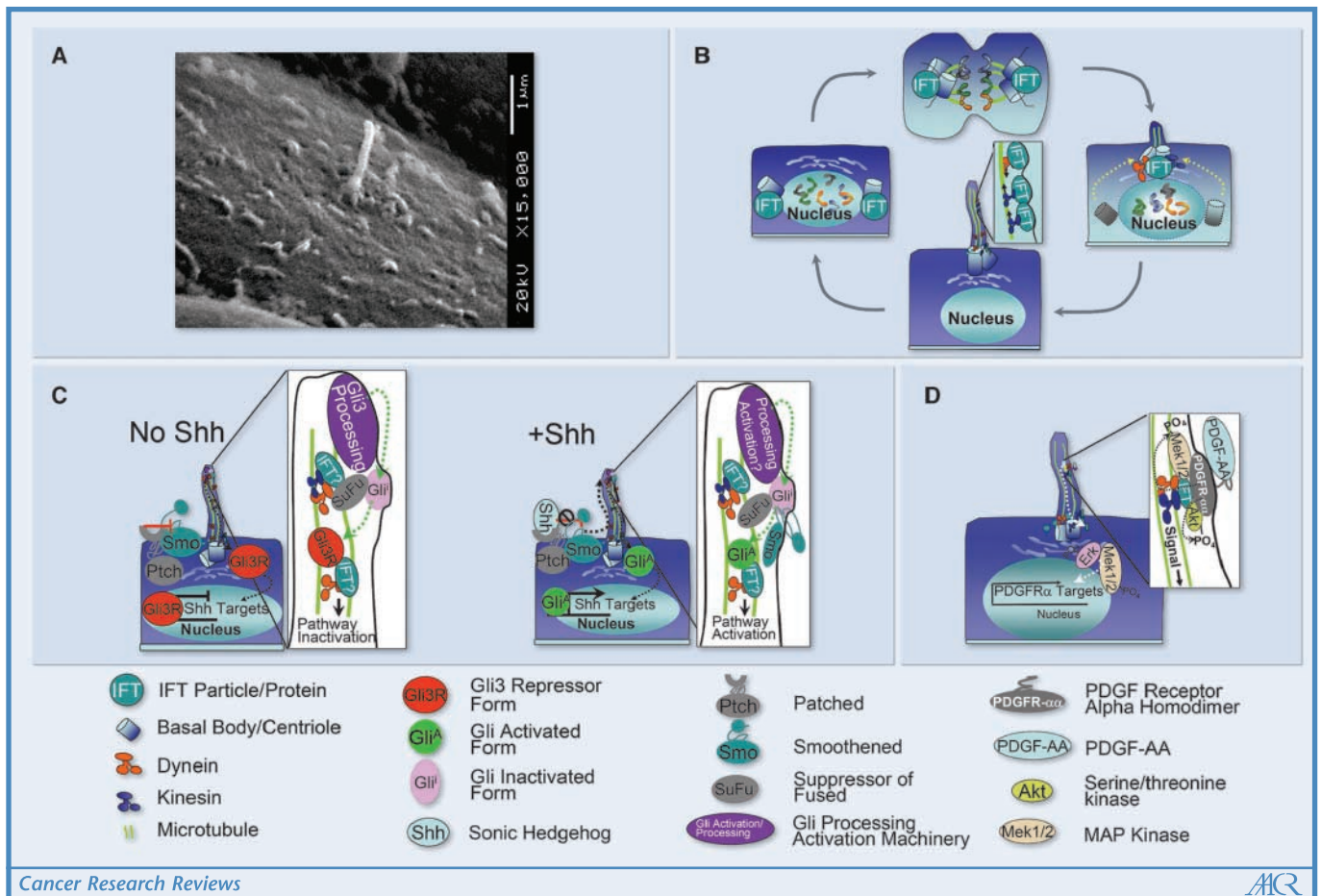
Figure 1. The primary cilium is an immotile sensory organelle that protrudes from most cells and is essential for ligand-induced signaling by the Shh and PDGFRα pathways in mammals. *A,* scanning electron micrograph of a primary cilium on a mouse limb-bud cell. *B,* the centrioles (barrels) of the cell assure proper chromosome segregation during mitosis, and in the quiescent phase of the cell cycle, the mother centriole (basal body) anchors the growing cilium to the plasma membrane, which is assembled by a process called IFT. *C,* upon binding of the Shh ligand to the Ptch1 receptor, repression of the Smo receptor on the cilium membrane is relieved and posttranslational modification of the Gli transcription factors within the cilium induces both their activator (Gli^A) and repressor functions. *D,* PDGF-AA binding to the PDGFRαα receptors on the cilium membrane induces phosphorylation and activation of the Akt and Mek1/2-Erk1/2 pathways, including phosphorylation of Mek1/2 within the cilium and at the basal body.

into the axoneme. The proteins necessary for assembling the cilium, for transporting receptors and channels to the cilium membrane, and for conveying sensory information from the cilium back to the cell body are moved bidirectionally along the axoneme in a process called intraflagellar transport (IFT; refs. 12, 13). Cytosolic proteins and vesicles containing transmembrane proteins dock at transition fibers at the base of the cilium and assemble into large complexes called IFT particles. These particles and associated cargo are transported anterograde (towards the cilium tip) along the microtubules of the cilium axoneme by the action of the kinesin-II motor complex, and retrograde (towards the cilium base) by the cytoplasmic dynein motor complex (Fig. 1*B*).

The Primary Cilium, Basal Body, Centrosome, and the Cell Cycle

The primary cilium is usually displayed on differentiated cells in G_0 (ref. 6; Fig. 1*B*). The primary cilium is anchored in the cell by the basal body located just beneath the plasma membrane, which

develops from the mother centriole of the centrosome in a manner that is coordinately regulated with the cell cycle (6, 14). As cells reenter the cell cycle, the cilium and basal body are disassembled, freeing the centrioles to function as the organizing center for the mitotic spindles, facilitating proper chromosome segregation during cell division (Fig. 1*B*). As cells become quiescent, the mother centriole forms the basal body and the primary cilium is again assembled (Fig. 1*B*). Further evidence that the primary cilium, basal body, and centrosome are united structurally and functionally comes from the observation that certain proteins associated with cilia-related diseases (e.g., inversin) are localized to all three structures (15).

The Mammalian Primary Cilium is a Mechanical and Chemical Sensor

It has been known for some time that defects in the motile cilia that line respiratory epithelia or brain ependymal cells have devastating effects on human health. However, primary cilia only recently became the focus of intense research and clinical

interest when it was discovered that the *Chlamydomonas IFT88* gene, which encodes a subunit of the anterograde IFT particle, and the orthologous mouse polycystic kidney disease gene, *Tg737*, are required for the assembly of flagella and cilia, respectively (16–18). Incredibly, it was then discovered that both dominant and recessive forms of polycystic kidney disease (ADPKD and ARPKD) in humans, as well as many syndromes that have cystic kidneys as a component of their disorder, are a result of defects in proteins that localize to the primary cilium, basal body, and/or centrosome (8–11, 15, 19, 20). Current data indicate that primary cilia in the kidney nephron act as mechanosensors that respond to flow-induced deflection of the axoneme by mediating a transient increase in intracellular calcium (21).

In addition to the mechanical sensing ability of primary cilia in the kidney, primary cilia in other tissues are biochemical sensors, as evidenced by the fact that they display receptors specific to the functions of the tissues in which they are located. Examples include the localization of the somatostatin receptor 3 (22) and 5-HT_6 serotonin receptor (23) specifically to neuronal cilia in certain parts of the brain. Other examples with direct relevance to cancer biology are the recent and exciting discoveries that multiple components of the Shh and PDGFRα pathways localize to primary cilia, and that cilia are essential for ligand-induced cell signaling by both pathways (Fig. 1*C* and *D*).

The Primary Cilium is Essential for Shh Signal Transduction

The Shh signaling pathway plays critically important roles during development, including establishment of the left-right body axis, neural tube closure and patterning, and formation of the limbs, teeth, pancreas, lungs, and hair follicles (24). Shh is a secreted ligand for a 12-membrane–spanning receptor called patched1 (Ptch1). The function of Ptch1 in the absence of Shh is to inhibit the activity of Smoothened (Smo), a seven-transmembrane signal transducer of the pathway (Fig. 1*C*). In the presence of Shh, Ptch1-mediated repression of Smo is eliminated, leading to activation of the pathway through the glioma (Gli) family of zinc-finger transcription factors. In vertebrates, three Gli proteins (Gli1, Gli2, and Gli3) are thought to exist in a microtubule-associated complex together with Suppressor of fused (Sufu), and possibly Costal-2 (Cos2) and Fused (Fu) homologues, although the functional importance of Cos2 and Fu remains unclear. Pathway activation is regulated through posttranslational modification of Gli proteins, with Gli1 and Gli2 primarily acting as transcriptional activators, whereas Gli3 has both activator and repressor functions (25).

A number of recent studies in mice have shown that primary cilia play an essential role in Shh signaling. Mice with mutations in either a subunit of the anterograde kinesin-II IFT motor (*Kif3a*), in two subunits of the retrograde dynein IFT motor (*Dync2li1* and *Dync2h1*), or in three IFT particle genes [*Ift88* (formerly *Tg737*), *Ift172*, and *Ift52*] each exhibit essentially the same mid-gestation lethal phenotype, consisting of dorsal-ventral neural tube patterning defects, randomized left-right axis specification, and polydactyly (26–32). Nodal and primary cilia in these mice are either completely absent or severely deformed. It was noted that the mutant phenotypes observed in mice lacking IFT function and cilia are consistent with alterations in the

Shh signal transduction pathway. Genetic epistasis experiments were then done that placed the IFT proteins downstream of the Ptch1 and Smo receptors and upstream of the targets of the Gli transcription factors (27, 30). Importantly, it was shown that the Smo receptor localizes to primary cilia in response to Shh, and that IFT function and cilia are required for Smo activity (31, 33). Moreover, it was shown that Gli1, Gli2, Gli3, and Sufu, a negative regulator of the pathway that binds the Gli proteins, all reside in the distal tip of the primary cilium (32). IFT proteins and cilia were shown to be required for both the transcriptional activator and repressor functions of the Gli proteins, including the proteolytic processing of full-length Gli3 to the repressor form and the transcriptional activity of Gli2 (29–32). Loss of Gli activator and repressor functions is consistent with the phenotypes exhibited by the IFT mutant mice (30). Loss of Gli activators, which play a major role in neural tube patterning, is in line with the loss of ventral neural tube cells in the mutant mice. Loss of Gli3 repressor function, which is more important in digit patterning, is consistent with the formation of extra digits. These data suggest that the primary cilium is a specialized organelle that contains the protein machinery required for the reception and transduction of Shh signaling activity. Primary cilia may be especially well-suited to fulfill this role, both in terms of the fact that they protrude into the extracellular milieu for the reception of secreted ligands, and from the perspective that they are a distinct domain within the cell that may serve as a location to concentrate and assemble the protein complex required for processing and activation of the Gli transcription factors (32).

These findings have significance to the understanding of certain basic mechanisms of cancer because overactivation of the Shh pathway is associated with numerous types of cancer, notably basal cell carcinoma, the most common type of cancer in humans, and medulloblastoma, the most common type of malignant childhood brain tumor. Thus, through a better understanding of the role that cilia play in Shh reception and Gli processing, novel approaches may emerge whereby unregulated overexpression of the Shh signaling pathway could be attenuated as an effective therapy in patients with numerous types of cancer.

The Primary Cilium is Essential for PDGFRαα Signal Transduction

Another significant advance showing the importance of cilia in cell signaling came from the work of Schneider and colleagues (34), who showed that the primary cilium is essential for signaling through PDGFRα homodimers (PDGFRαα). The platelet-derived growth factor (PDGF) signaling pathway is essential for normal embryogenesis, inflammation, and wound healing, and unregulated activation of the pathway is associated with numerous disorders, including carcinogenesis. The PDGF pathway consists of four ligands (PDGF-A, -B, -C, and -D) and two receptors (PDGFRα and PDGFRβ). The ligands form homodimers (and AB heterodimers) and bind to two receptors simultaneously, resulting in the activation of the receptors. The affinities of the receptors for the various PDGF homodimers differ. For example, PDGF-AA only binds to PDGFRαα, whereas PDGF-BB binds all three dimeric combinations of the two receptors. Upon ligand binding, receptor dimerization and autophosphorylation on specific tyrosine residues in the cytoplasmic domain of the receptors facilitates the binding of intracellular signaling molecules

to these phosphotyrosines and initiates downstream signaling cascades, which include the phosphoinositide-3-kinase/Akt and Raf/MEK/ERK pathways (4, 35).

Schneider et al. (34) explored the connection between cilia and PDGF signaling based on the prior observations that cilia are usually formed on growth-arrested cells, and PDGFRα is preferentially expressed in growth-arrested cells. They showed that PDGFRα localizes to the primary cilia of growth-arrested NIH/3T3 mouse fibroblasts, whereas PDGFRβ does not, but instead occurs in clusters along the cell surface. Immunoblots showed that both PDGFRα and PDGFRβ were present in interphase cells prior to the development of cilia, but only PDGFRα showed increases in protein levels that correlated with the development and elongation of cilia. PDGF-AA stimulation of cells resulted in the activation of PDGFRαα, followed by phosphorylation and activation of the Akt and Mek1/2-Erk1/2 pathways (Fig. 1D). Interestingly, it was shown that phosphorylation of Mek1/2 occurred within the cilium and at the basal body. These authors showed that growth arrest of fibroblasts from $Tg737^{orpk}$ ($Ift88^{Tg737Rpw}$) mutant mice failed to induce the up-regulation of PDGFRα. In addition, PDGF-AA stimulation of the mutant cells failed to activate PDGFRαα and the Mek1/2-Erk1/2 pathway, and reentry of the cells into the cell cycle was impaired. Thus, cilia are essential for PDGF signal reception and pathway activation through PDGFRαα. Localization and activation of PDGFRα signaling, but not PDGFRβ in the primary cilium, is likely to add yet another layer of specificity to the differential roles of PDGFs and their receptors in a myriad of cellular functions.

Like the Shh pathway, PDGFRα signaling influences multiple cellular responses including chemotaxis, proliferation, and apoptosis, and has directly been linked with tumor formation in humans, especially in gliomas and gastrointestinal stromal tumors (4, 35). Thus, the discovery that primary cilia have an essential role in regulating PDGFRα signaling may provide novel insights into the pathogenesis of these tumors.

The Primary Cilium as a New Target for Cancer Therapeutics?

These recent discoveries on the essential role of the primary cilium in regulating cellular responses to Shh and PDGF signaling, together with the importance of these pathways in cancer biology, suggest that further investigations into the sensory functions of the primary cilium will provide significant advances in our understanding of certain basic mechanisms of carcinogenesis. Genes and proteins involved in the structure or function of the primary cilium may represent new targets for small-molecule inhibitors, small interfering RNAs, or antibody therapeutics. Additionally, many of the proteins required for ciliogenesis also localize to the centrioles during mitosis. Therefore, defects in these proteins may lead to the development of cancer through alterations in the cell cycle or in chromosomal stability, independent of the pathways discussed here. Given that the cilia proteome consists of approximately 300 to 500 proteins (36, 37) and the biological functions of most of them are unknown, it is clear that there are many exciting discoveries yet to come on the role of the primary cilium in cell signaling and cancer.

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