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Constructing and deconstructing roles for the primary cilium in tissue architecture and cancer

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Abstract

Primary cilia are exquisitely designed sensory machines that have evolved at least three distinct sensory modalities to monitor the extracellular environment. The presence and activation of growth factor, morphogen, and hormone receptors within the confines of the ciliary membrane, the intrinsic physical relationship between the ciliary axoneme and the centriole, and the preferential assembly of primary cilia on the apical surfaces of tissue epithelia highlight the importance of this organelle in the establishment and maintenance of tissue architecture and homeostasis. Accordingly, recent studies begin to suggest roles for these organelles in oncogenesis and tumor suppression. Here, we review the sensory properties of primary cilia, assess the “history” of the primary cilium in cancer, and draw upon recent findings in a discussion of how the primary cilium may influence tissue architecture and neoplasia.

Introduction

Primary cilia are assembled by many cell types within the human body (<http://www.bowserlab.org/primarycilia/cilialist.html>) and in some cases during select stages of development (Marion et al., 2009). Their unique topology provides a highly specialized surface area which may be fully dedicated to the detection, transformation, and relay of external cues to the cell body. In addition, there are likely to exist important relationships between the cilium and the cell cycle, as would be suggested by the dynamic assembly and resorption patterns seen during the cell cycle (Fonte et al., 1971; Tucker et al., 1979) and by the intrinsic relationship between cilium and centriole (Satir and Christensen, 2007). Accordingly, defective cilium assembly or function can lead to pleiotropic disorders of tissue architecture and proliferation, including polycystic kidney disease (Pazour et al., 2000; Qin et al., 2001; Yoder et al., 2002), Bardet Biedl Syndrome (Ansley et al., 2003; Nachury et al., 2007), and Alstrom Syndrome (Hearn et al., 2005; Li et al., 2007). While there have been few thorough studies of the behavior of this organelle in the setting of cancer interest in the relationship between primary cilia and oncogenesis is growing. Here, we review the remarkable sensory properties of primary cilia, the classes of receptor complexes that are found within the ciliary membrane, and how primary cilium function and dysfunction influences tissue homeostasis, architecture, and potentially, the development of cancer.

Sensory modalities

Primary cilia have evolved distinct structural properties and at least 3 distinct sampling modalities to detect and transmit a wide variety of different stimuli to the interior of the cell. The primary cilium has the capacity to sense flow and mechanical stress via classes of mechanosensory calcium channels present in the ciliary membrane (Praetorius and Spring, 2001; Yoder et al., 2002), low-abundance ligands via resident, non-cycling receptors (Huang et al., 2007), and changes in ligand concentrations by utilizing intraflagellar transport (IFT),

or other transport machinery, to provide continuous sampling (Huang et al., 2007; Rohatgi et al., 2007). Additional mechanisms for 'tuning' might occur through regulated delivery of ciliary signals at the level of retrograde trafficking or cilium resorption. Together, these properties render the primary cilium exceptionally well equipped to enforce tissue homeostasis and to stimulate adaptive changes in tissue architecture.

Flow and mechanosensation

The ability to detect and monitor flow rates and shear forces permits the function and adaptation of kidney tubules and the ducts of liver and pancreas. The conduction of fluids from one location to another by these cellular tubes is life-sustaining and must be preserved in the face of occlusion. How then, might these systems adapt to preserve flow should blockage arise? Ideally, a luminal detection system would be in place to detect diminished flow rates and increase tubule caliber. The primary cilium contains flow-responsive calcium channels that, when activated, flux calcium ions to the cytoplasm (Praetorius and Spring, 2001). The many functions of intracellular calcium ions include stimulation of cytoskeletal remodeling and cell division, two activities which can lead to increased tubule caliber. In the setting of constant unidirectional flow, such as that of urine in the nephron, the flow-responsive calcium channels on the upstream face of the cilium, those that are constantly subjected to lateral tension and shear forces, are likely silent secondary to stimulation-dependent desensitization (figure 1). In contrast, those of the downstream face are likely silent due to lack of lateral tension and exposure to flow (figure 1). Thus, during continual unidirectional flow, the primary cilia of the nephron are inactive with regard to calcium flux. In the setting of occlusion, fluid back up, reversal of flow, and increased fluid pressure within the system would reverse cilium orientation and increase lateral tension along the newly upstream face of the ciliary membrane, triggering calcium influx (figure 1). Depending on context, such an event may lead to cellular lengthening and cell division. In this way, the cilium may increase tubule caliber and restore flow in the face of occlusion. Similarly, stimulation of the primary cilia of the vasculature leads to calcium influx and to nitric oxide production, two second messengers that increase vessel caliber (AbouAlaiwi et al., 2009). In accord with these concepts, the loss of the regulatory environment provided by the cilium results in unabated calcium influx in renal tubule cells and to tissue pathology otherwise seen following irreversible tubule occlusion (Pazour et al., 2000; Cano et al., 2004; Zhang et al., 2005).

Low abundance ligands

In many circumstances, trace amounts of ligand are utilized to coordinate homeostatic signaling. Receptors, including growth factor and hormone receptors, are often highly concentrated within the ciliary membrane (Ma et al., 2005; Schneider et al., 2005), permitting the accumulation of ligand-receptor complexes on the ciliary membrane. Further, there is evidence to suggest that the cilium can immobilize certain receptor populations within its membrane while subjecting others to continuous treadmilling (Huang et al., 2007). The ability to concentrate and immobilize receptors within the ciliary membrane maximizes sensitivity and at the same time distances high concentrations of catalytic receptor complexes from important cytoplasmic effectors, minimizing the potential consequences of aberrant receptor 'firing'. To activate their cytoplasmic or nuclear effectors, activated ciliary receptors must be delivered to the cell body by IFT, other ciliary trafficking systems, or cilium resorption, offering additional steps that may increase fidelity and minimize aberrant signal transduction (figure 2).

Concentration shifts

A third sensory modality made available by the primary cilium relates to IFT and the presence of receptors which cycle in and out of the ciliary membrane, permitting continuous sampling

of the extracellular environment (Huang et al., 2007; Rohatgi et al., 2007). Here, the rate-of-receipt of occupied or activated receptor complexes relayed from the cilium and detected in the cell body can serve to adjust or activate signaling pathways important for adaptive or developmental programs (Figure 2). Intriguingly, IFT is quite dynamic and the rate of entry and exit of IFT particles in and out of the cilium can vary markedly (Pan and Snell, 2005), providing for continuous tuning of ciliary sampling rates according to ligand and physiological context.

Ciliary Receptors

Clearly, the structural and biochemical features of the primary cilium convey a marked capacity for sensory activities. What types of receptors take advantage of the flexibility and fidelity conveyed by this unique signaling platform? Growth factor, morphogen, odorant, and hormone receptors, ion channels, and multipass G-protein - coupled receptors have all been localized to the ciliary membrane (Marshall and Nonaka, 2006). At present, due to the lack of a primary cilium preparation of high purity and yield, the full complement of ciliary receptor modules has yet to be enumerated. However, targeted analyses have yielded an already remarkable diversity.

Growth Factor Receptors

Growth factor receptors are coupled to mitogenic signaling cascades and ion channels which regulate cell growth and proliferation. Both epidermal growth factor receptor (EGFR) and platelet-derived growth factor receptor- α (PDGFR- α) are known to localize to the ciliary membrane (Schneider et al., 2005; Ma et al., 2005). Following serum starvation, PDGFR- α is present nearly exclusively (Schneider et al., 2005) and EGFR markedly enriched (Ma et al., 2005) in the ciliary membrane. PDGFR- α ligation leads to the rapid appearance of phosphotyrosine and phospho-Mek1/2 along the length of the cilium (Schneider et al., 2005). In the absence of the primary cilium, PDGFR- α fails to accumulate in any membrane compartment (Schneider et al., 2005). Together, these findings suggest that the cilium functions as the exclusive site of PDGFR- α activation. In addition, the appearance of phospho-Mek1/2 in the cilium suggests that intermediate components of the PDGFR signaling cascade, such as Ras, Raf, and PKC, are also present within the primary cilium.

While EGFR too has been shown to localize to primary cilia (Ma et al., 2005), the significance of its ciliary localization is unclear at present. At a minimum, there is evidence to suggest that stimulation of cilium-localized EGFR leads to calcium flux via the polycystin calcium channel (Ma et al., 2005). Whether downstream kinase activation occurs within the cilium or whether ciliary activation of the EGFR is sufficient to induce cytoplasmic signal transduction remain to be seen however. In this regard, it would be interesting to learn whether ciliary EGFR signaling induces cellular responses distinguishable from those of plasma membrane - associated signaling.

While other canonical growth factor receptors have not yet been found within primary cilia, fibroblast growth factor has been shown to modulate cilium length (Neugebauer et al., 2009). It will no doubt be of interest to learn the complete repertoire of ciliary growth factor receptors and to develop a better understanding of how these proteins are trafficked to and within the cilium.

Morphogens

Morphogens such as those of the Wnt and Hedgehog (Hh) families mediate important developmental switches and can influence cell fate and nascent tissue architecture over long distances. While not appreciated for many years, the discovery and biochemical

characterization of IFT led rapidly to the realization that primary cilia have central roles in mammalian morphogen signaling (Huangfu, 2005).

In a phenotypic screen designed to identify genes important for hedgehog signaling in mice, Anderson and coworkers found that mutation of IFT172 gave rise to neural tube and other defects seen in hedgehog mutants (Huangfu, 2005). Subsequent work from many additional groups has shown that Smoothened (Smo), Patched (Ptch), and Gli family transcription factors all localize to the primary cilium and seem to traffic dynamically in and out of the organelle in a Hh dependent manner (Corbit et al., 2005; Haycraft et al., 2005; Rohatgi et al., 2007). Defects in cilium assembly prevent Gli processing and activation of Gli activator and repressor functions in response to Hh signals (Liu et al., 2005; Haycraft et al., 2005). A current model of ciliary Hh signaling posits that Hh-Ptch interactions relieve Ptch-mediated inhibition of Smo, leading to ciliary Smo accumulation and to the activation of Gli processing and function (Rohatgi et al., 2007). While the molecular details of the mechanisms of ciliary Hh-pathway activation remain to be fully elucidated, they clearly evidence the remarkable dynamics of regulated membrane traffic occurring between the primary cilium and other cellular compartments. Important issues to be further addressed are whether alternative pathways of Gli processing might function in the absence of a primary cilium *in vivo*, which transport systems traffic Hh-pathway components to and within the primary cilium, and why primary cilia are required to modulate Hedgehog signaling in mammals but not lower organisms.

While there has been a clear relationship established between the primary cilium and Wnt signaling, the receptors for soluble Wnts have not yet been localized to the primary cilium, though they have been found to concentrate at its base in close proximity to the basal body (Ross et al., 2005; Corbit et al., 2008). However, both APC and beta-catenin, a negative regulator and effector of canonical Wnt signaling, respectively, have been localized to the cilium. Interestingly, the loss of the primary cilium via mutations affecting the basal body or IFT lead to enhanced activity along the Wnt pathway, nuclear accumulation of beta-catenin, and to increased cellular responsiveness to soluble Wnts (Cano et al., 2004; Corbit et al., 2008; Jonassen et al., 2008). Accordingly, the expression of Wnt ligands are increased markedly, likely via an autocrine mechanism, in cilium assembly mutants (Jonassen et al., 2008). Similarly, the loss of the BBSome subunit Bbs1 leads to an increase in beta-catenin signaling and to potentiation of Wnt responsiveness (Ross et al., 2005). Together, these findings have led to speculation that ciliary defects cause PKD cyst formation through increased Wnt signalling.

Hormone and G-protein-coupled receptors

While the relationship between the cilium and growth factor and morphogen receptors is becoming clear, little is known regarding the influence of ciliary localization on signaling by the odorant and hormone receptors that are found there. Serotonin, somatostatin, leptin, and 'odorant' receptors are all constituents of the ciliary membrane as are a number of other uncharacterized GPCRs (Marshall and Nonaka, 2006).

Tissue Homeostasis and Architecture

The important relationship between primary cilia and development can be deduced from the complement of signaling receptors found within the ciliary membrane, the stimulus-dependent trafficking of morphogen receptors and their downstream effectors in and out of the primary cilium, the deregulation of growth and developmental pathways in cilium assembly mutants, and the remarkable pleiotropism of heritable developmental defects that are seen in the setting of cilium dysfunction in humans.

As described earlier, the sensory capacities of the primary cilium provide means to shape tissue architecture in response to environmental challenge and developmental cues. In humans, heritable mutations in genes required for cilium assembly or in those encoding ciliary receptors leads to disordered epithelial proliferation and to the kidney, pancreas, and liver cysts seen in polycystic kidney disease. Mutations affecting cilium function also lead to Bardet Biedl and Alstrom syndromes, two pleiotropic disorders encompassing cyst formation, dilated cardiomyopathy, short stature, type II diabetes, morbid obesity, hypertension, polydactyly, mental retardation, and skeletal abnormalities, and to a host of other rare heritable disorders of development (Sharma et al., 2008). What general concepts may explain how cilium dysfunction might lead to such developmental defects?

Growth-promoting ligand receptors which are normally sequestered within the primary cilium accumulate in other cellular compartments or are destabilized in cilium assembly mutants (Schneider et al., 2005; Lin et al., 2003; Ma et al., 2005). EGFR is known to accumulate within primary cilia and the loss of Kif3a, a component of the anterograde IFT motor complex, prevents cilium assembly and leads to EGFR accumulation and persistent MAPK activity (Lin et al., 2003; Cano et al., 2006). It is likely that, in addition to participating in calcium signaling, growth factor receptors such as EGFR and PDGFR- α are sequestered to the cilium during quiescence to prevent aberrant signaling, to couple receptor activation to one or more of the sensory modalities of the cilium, and to impose a requirement for cilium resorption or retrograde ciliary trafficking on the activation of cytoplasmic and nuclear targets. Similarly, in the absence of a primary cilium, polycystin 2 accumulates in the apical plasma membrane of kidney tubule cells, leading to unabated and deregulated calcium signaling (Siroky, 2006). Together, these findings suggest that the loss of the cilium can lead to redistribution of its membrane effectors and subsequently, to aberrant signal transduction. It is tempting to speculate that signaling via this mechanism may account for the increased levels of c-Myc that are also seen in cilium assembly mutants (Lin et al., 2003; Jonassen et al., 2008).

The cilium may also function to coordinate the downregulation of certain signaling molecules. Proteasomes localize about basal bodies and centrosomes and a number of ciliary proteins, including beta-catenin, are known to be downregulated via proteasomal degradation (Aberle et al., 1997; Fabunmi et al., 2000; Gerdes et al., 2007). Indeed a recent study suggests that the loss of a number of BBS proteins leads to the stabilization of beta-catenin in part by disrupting proteasome function (Gerdes et al., 2007). It is possible that the cilium provides a conduit for trafficking to the proteasome and, in this way, the loss of the cilium could lead to persistent signaling due to the accumulation of proteasome targets such as beta-catenin. Such a mechanism could contribute to the increase in Wnt signaling seen in cilium assembly mutants.

Overall, while it is clear that defects of cilium assembly or ciliary receptors lead to dramatic alterations in tissue architecture, the specific mechanisms involved have yet to be revealed and we are left to speculate. It is intriguing however, that the loss of the primary cilium seems to recapitulate architectural phenotypes that might be expected were certain of the known receptors of its membrane to signal continuously.

Cancer

There are compelling reasons to believe that primary cilia can stimulate or enhance cancer development. Because primary cilia are required for Hedgehog to activate downstream Gli transcription factors, the loss of this organelle should repress the development of hedgehog dependent cancers bearing mutations that lie upstream of the Glis but not affect the development of tumors bearing activating mutations in the Gli transcription factors themselves. Indeed, a pair of recent studies (Han et al., 2009; Wong et al., 2009) have shown that the development of Smo-dependent neoplasms requires the presence of primary cilia while those

that depend on mutant Gli2 are enhanced by mutations that preclude primary cilium assembly. In the case of medulloblastoma, the presence of cilia amongst a group of human tumors correlated with the presence of Wnt or Hedgehog pathway mutations, in accord with roles for the cilium in modulation of these pathways.

While the primary cilium can modulate the development of hedgehog dependent neoplasms, the relationship between this organelle and the development of more common malignancies is less clear. The loss of genes encoding proteins required directly for cilium assembly leads to epithelial hyperplasia, metaplasia, and cystic disease; defects in cell and planar cell -polarity; persistent Wnt, TGF-beta, and MAPK signaling; EGFR and c-Myc accumulation; and deregulated Hh signaling (Pazour et al., 2000; Lin et al., 2003; Cano et al., 2004; Cano et al., 2006; Corbit et al., 2008; Jonassen et al., 2008). Further, the loss of the cilium may remove requirements for cilium resorption, basal body disassembly, and receptor transport steps from the cell division cycle (figure 2). As such, it would be expected that loss or dysfunction of the cilium may stimulate the development of more common epithelial malignancies.

What then, is the fate and significance of the primary cilium during cancer development? No clear picture has yet emerged. The majority of instances in which this topic is treated in the literature occur as reports of pathological oddities rather than careful analyses involving significant numbers of patient specimens. While it has been suggested that the loss of the primary cilium is a well known consequence of cellular transformation (Plotnikova et al., 2008; Pugacheva et al., 2007), an extensive review of this topic (Wheatley, 1995) states, "it is already well known that a wide variety of tumours possess cells with primary cilia...several cases have been reported of malignant cell types apparently producing cilia after malignant transformation when their 'normal counterparts' were supposedly unciliated, as in the case of hepatocytes of the LI-IO line," and, "...transformed variants of the otherwise well-behaved cell line, 3T3 still exhibit, however, the same high incidence of ciliation at confluency as the progenitor, untransformed 3T3 line."

An additional problem relates to the significance of a loss of primary cilia, were it to occur as a regular feature of neoplastic transformation. Primary cilia exhibit dynamic patterns of assembly and disassembly as they progress through the cell cycle (Fonte et al., 1971) and there exist examples of cell types in which ciliation occurs only during quiescence (Dingemans, 1969) and others in which cilia are present during interphase (Fonte et al., 1971; Tucker et al., 1979). As cilium resorption can thus occur as a physiologic consequence of cell cycle progression, any loss of this organelle in cancer may merely represent the enhanced rates of cell division that are seen in this setting. This issue has been almost completely ignored by recent studies, including those attempting to link mutations of the VHL tumor suppressor to defects in ciliogenesis (Lutz, 2006; Esteban, 2006) and more recent studies examining the presence of cilia in clinical medulloblastoma and basal cell carcinoma specimens. Indeed, careful studies demonstrate that VHL deletion alone has no effect on the ciliation of renal epithelial cells and that PTEN or GSK3-beta must also be deleted to block cilium assembly (Thoma et al., 2007; Frew et al., 2008). While the potential involvement of proliferative deciliation was not addressed for GSK3-beta/VHL co-deletion, PTEN/VHL codeletion lead to a 5 to 6 -fold increase in proliferation (Frew et al., 2008). Thus, it is very possible that the loss of primary cilia in these settings is a physiological consequence of enhanced proliferation.

To address these issues, we recently examined the fate of the primary cilium in the setting of pancreatic ductal adenocarcinoma (PDA) (Seeley et al., 2009). Here, in 17 patients and in murine developmental models, we found that primary cilia were completely absent from the neoplastic epithelium (figure 3) (Seeley et al., 2009) but not the stroma of PDA lesions (ES Seeley, unpublished observation). The majority of cases of PDA are thought to initiate via the formation of preinvasive neoplasms termed Pancreatic Intraepithelial Neoplasia (PanIN) and

we were likewise unable to identify a single ciliated neoplastic epithelium in any of these types of lesions. In agreement with several prior studies, 97% and 60% of the neoplastic epithelial cells in the earliest appearing PanINs and in PDA, respectively, were quiescent as assessed by Ki67, PCNA, and BrdU labelling. In contrast, the epithelia of chronic pancreatitis and acinar to ductal metaplasia were not associated with primary cilium loss but were instead associated with highly elongated cilia.

Interestingly, when oncogenic Kras was expressed in all pancreatic lineages, primary cilia were lost only from neoplastic epithelium and not from untransformed islets, ducts, centroacinar cells, or acinar-ductal metaplasms. Finally, while confluence and serum deprivation -induced quiescence did not restore cilium assembly in murine PDA cells, the addition of PI3 kinase inhibitors induced robust ciliation in both contexts. We draw several conclusions from this study. One, a pathologic and reversible suppression of cilium assembly occurs as a constant feature of pancreatic cancer. Two, primary cilia depletion can occur as a consequence of oncogenic signaling in the setting of cell cycle arrest and independent of dominant mutations in genes required for organelle assembly. Three, cilium depletion is likely to represent an intrinsic feature of pancreatic epithelium transformation. However, key questions remain unanswered. For example, it is not known whether the loss of genes required for cilium assembly might enhance PDA development nor how the assembly of the organelle is actually suppressed in PDA.

However, akin to the studies of Wong and Han, the pattern of primary cilium loss in PDA provides predictions regarding the influence of Hedgehog and Wnt signaling in this malignancy. Primary cilia are present on stromal cells (E. Seeley, unpublished), absent from the cancerous epithelium, and are required for Hh stimulation of the Hedgehog pathway (Haycraft et al., 2005). Thus, Hedgehog pathway activation should occur only in the stromal compartment of PDA. Indeed, several recent studies have shown that, while Hh is expressed in the neoplastic epithelium of PDA, pathway activation seems to occur solely in the stroma (Theunissen and de Sauvage, 2009). In contrast, the fact that cilium dysfunction leads to enhanced Wnt activation and secretion (Cano et al., 2004; Corbit et al., 2008; Jonassen et al., 2008) predicts that Wnt signaling would be enhanced in both the stroma and neoplastic epithelium of PDA. This too has recently been demonstrated (Pasca di Magliano et al., 2007; Wang et al., 2009). While the significance of these geographical patterns of activity and their potential interdependence remains unclear, they fit well with the pattern of cilium loss in PDA.

Summary

In all, it is not difficult to imagine that primary cilium dysfunction may play a role in the development of malignancies which arise from ductular epithelia such as those of the liver, kidney, prostate, and pancreas. Either directly or indirectly, the assembly and function of this organelle is likely, like other organelles, to require the function of several hundred gene products. Thus, a great number of transcriptional perturbations or mutations or combinations thereof could lead to frequent cilium dysfunction in the setting of cancer. From this perspective, it would be perhaps surprising if primary cilium function was not frequently altered in some way during oncogenesis. In this regard, it will be particularly interesting to learn if cilium integrity is required only for cancers which depend on hedgehog signaling or instead, whether the genetic diversity of cancers is reflected by distinct patterns of ciliation across the spectrum of human malignancy.

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Figure 1.

Figure 2.

Figure 3.