

Chapter 8

Primary Cilia and Brain Cancer

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Contents

8.1	Introduction.....	210
8.2	Primary Cilia and Cell Cycle Progression.....	210
8.3	Primary Cilia and Signaling Pathways.....	212
8.3.1	Hedgehog Signaling.....	212
8.3.2	Wnt Signaling.....	213
8.3.3	RTK Signaling.....	214
8.3.4	Notch Signaling.....	215
8.4	Primary Cilia and Medulloblastoma.....	216
8.5	Primary Cilia and Other Brain Cancers.....	219
8.6	Conclusion.....	221
	References.....	222

Abstract Cell-extrinsic signals and intrinsic cell cycle regulators strictly control proliferation. Cancers develop from a cell that escapes these tight controls and proliferates unrestrictedly. The primary cilium critically controls proliferation by mediating cell-extrinsic signals and regulating cell cycle entry. Accordingly, recent studies showed that defective cilia can either promote or suppress cancers, depending on the cancer-initiating mutation, and that presence or absence of primary cilia is associated with specific cancer types. These novel findings suggest that primary cilia play central but distinct roles in different cancer types, opening up a completely new avenue of research to understand the biology and treatment of cancers.

Keywords Primary cilia • Cell cycle • Hedgehog • WNT • NOTCH • Receptor tyrosine kinase • Cancer

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Outline

In this chapter, we review evidence showing that primary cilia play important roles in cell cycle entry and multiple signaling pathways, and we discuss how these roles of primary cilia could contribute or inhibit brain cancer formation. First, we review the dual and opposing roles of primary cilia in medulloblastoma development, then discuss possible roles of primary cilia in other brain cancers focusing on diffuse intrinsic pontine glioma, glioblastoma multiforme, and cancer stem cell. Lastly, we discuss the potential of primary cilia in diagnosis and treatment for brain cancers.

8.1 Introduction

The primary cilium is at the crossroads of cell cycle progression and cellular signaling pathways. Cell cycle progression regulates assembly and disassembly of primary cilia, and primary cilia in turn regulate cell cycle entry (Rieder et al. 1979; Tucker et al. 1979; Kim et al. 2011b; Li et al. 2011). Primary cilia participate in multiple signaling pathways that control cell proliferation, differentiation, migration, polarity, and metabolism, deregulation of which are closely linked to oncogenesis (Huangfu et al. 2003; Schneider et al. 2005; Simons et al. 2005; Zhu et al. 2009; Boehlke et al. 2010; Berbari et al. 2011; Ezratty et al. 2011). Thus, it is reasonable to suspect that normal or abnormal functions of primary cilia may contribute to oncogenesis. Indeed, recent studies showed that primary cilia play fundamental roles in the development of basal cell carcinoma, the most common cancer in Caucasians, and medulloblastoma, the most common brain cancer in children (Han et al. 2009; Wong et al. 2009). Surprisingly, primary cilia are either required or suppressive for oncogenesis in mice, depending on the cancer-initiating mutation. Furthermore, the presence or absence of primary cilia is associated with specific cancer types in humans (Wheatley 1995; Han et al. 2009; Schraml et al. 2009; Seeley et al. 2009; Wong et al. 2009; Yuan et al. 2010; Kim et al. 2011a), suggesting that primary cilia can either promote or suppress human cancers as well. Therefore, understanding the mechanisms by which this intriguing organelle functions in normal and cancerous cells will reveal oncogenic mechanisms that were not apparent previously and will unmask new therapeutic approaches.

8.2 Primary Cilia and Cell Cycle Progression

The primary cilium contains a unique microtubule cytoskeleton called the axoneme, a ring of nine microtubule doublets (9+0) that runs longitudinally through the organelle. The axoneme grows from the basal body docked with the cell membrane. During cell division, the basal body detaches from membrane and transforms into the mother centriole to form the centrosome that organizes the mitotic spindle.

Thus, ciliogenesis is tightly regulated throughout the cell cycle; proliferating cells disassemble cilia before mitosis to release the basal body and reassemble them after mitosis (Rieder et al. 1979; Tucker et al. 1979). Some cells also resorb cilia upon entry into the S phase (Tucker et al. 1979). Consistent with this regulation, Aurora kinase A, a centrosomal kinase that regulates mitotic entry, becomes activated at the basal body as cells enter the S phase and mitosis and triggers ciliary disassembly (Pugacheva et al. 2007). As a cell exits mitosis, CP110, a distal centriolar protein that inhibits ciliogenesis, disappears from the mother centriole, allowing ciliogenesis to occur (Spektor et al. 2007). Cyclin-dependent kinase inhibitors also influence ciliogenesis in cultured cells; knockdown of *p16^{INK4a}* positively influences ciliogenesis, and knockdown of *p15^{INK4b}* negatively regulates it (Bishop et al. 2010; Kim et al. 2010a). However, mutant mice defective for *p16^{INK4a}* and/or *p15^{INK4b}* do not display developmental defects associated with defective cilia. Remarkably, recent studies showed that this cell-cycle-dependent organelle is not passively linked with the cell cycle but actively regulates cell cycle progression (Bielas et al. 2009; Jacoby et al. 2009; Kim et al. 2011b; Li et al. 2011).

One of the first pieces of evidence supporting the role of primary cilia in cell cycle progression came from studies on the *inositol polyphosphate-5-phosphatase E (INPP5E)* gene mutated in two cilia-associated diseases, Joubert and MORM syndromes (Bielas et al. 2009; Jacoby et al. 2009). INPP5E, which hydrolyzes the 5-phosphate of phosphatidylinositol 3,4,5-triphosphate and phosphatidylinositol 4,5-bisphosphate, localized exclusively at primary cilia; thus, mutation in *INPP5E* caused the ciliary membrane to accumulate these lipids, which play important roles in signaling pathways and membrane trafficking. Notably, *INPP5E* mutation did not affect assembly of cilia but accelerated both ciliary disassembly and S-phase entry after serum stimulation. Previous work showed that overexpression of *INPP5E* caused cell cycle arrest (Kisseleva et al. 2002). These studies suggest that ciliary disassembly can affect S-phase entry. More direct evidence supporting this notion came from the following two studies.

In one study, Sung and colleagues (Li et al. 2011) found that Tctex-1, a light chain subunit of cytoplasmic dynein, plays critical roles in ciliary disassembly and cell cycle progression. Knockdown of Tctex-1 inhibited both ciliary disassembly and S-phase entry that occur after serum addition to serum-starved NIH3T3 and RPE cells. Importantly, Tctex-1 knockdown did not affect S-phase entry in cells lacking primary cilia, indicating that failure to disassemble cilia underlies the blockage of S-phase entry. This inhibition was independent of the function of Tctex-1 as a cytoplasmic dynein component. Serum stimulation induced phosphorylation of Tctex-1 at threonine 94 (T94), leading to its dissociation from the dynein complex and accumulation at the transition zone between the basal body and the ciliary axoneme. The knockdown effect was rescued by wild-type and phosphorylation-mimicking Tctex-1 (Tctex-1^{T94E}), but to a lesser extent by non-phosphorylatable Tctex-1^{T94A}, which binds dynein. Furthermore, Tctex-1^{T94E} accelerated ciliary disassembly and S-phase entry even in the absence of serum stimulation. *In vivo*, Tctex-1 is selectively enriched in proliferating neural progenitors. Remarkably, knockdown of Tctex-1 in radial glia, the neural progenitors in the embryonic brain, caused them to exit from the cell cycle and differentiate into neurons prematurely, whereas overexpression of Tctex-1^{T94E}

shortened the G1 phase, accelerated S-phase re-entry, and increased the proliferating progenitor cell population. These data indicate that the primary cilium blocks S-phase entry and cells use Tctex-1 to overcome this blockage.

In the other study, Tsiokas and colleagues (Kim et al. 2011b) found that Nde-1, a centrosomal protein, critically regulates cilia length and S-phase entry. Knockdown of Nde-1 in NIH3T3 and RPE cells lengthened cilia and delayed S-phase entry after serum stimulation. Importantly, similar to Tctex-1, the delay in S-phase entry upon Nde-1 knockdown was dependent on the presence of primary cilia; there was no delay in S-phase entry in the absence of primary cilia. Furthermore, expression of constitutively active form of Rab8a, which lengthens primary cilia independently of Nde-1, also inhibited S-phase entry, confirming that lengthened cilia delay S-phase entry. *In vivo*, knockdown of *Nde-1* in zebrafish caused lengthening of primary cilia and defective proliferation in Kuffer's vesicle. Interestingly, mutations in *Nde-1* cause microcephaly in both humans and mice (Feng and Walsh 2004; Alkuraya et al. 2011; Bakircioglu et al. 2011), which may be partly due to premature cell cycle exit and differentiation of neural progenitors that have abnormally long cilia. Taken together, these studies revealed that primary cilia regulate cell division as a barrier blocking S-phase entry raising the possibility that primary cilia negatively regulate oncogenesis.

8.3 Primary Cilia and Signaling Pathways

Studies over the past decade have established primary cilia as a signaling hub for multiple signaling pathways, including Hedgehog (Goetz and Anderson 2010), Wnt (Wallingford and Mitchell 2011), receptor tyrosine kinases (RTKs) (Christensen et al. 2012), and Notch signaling (Ezratty et al. 2011). These signaling pathways control a myriad of cellular processes, including proliferation, differentiation, migration, polarity, and metabolism, all of which play critical roles in development, homeostasis, and oncogenesis. Here we briefly discuss the role of primary cilia in these signaling pathways.

8.3.1 Hedgehog Signaling

Forward genetic studies in mice have shown that Hedgehog signaling requires the primary cilium for both activation and repression of the pathway (Huangfu et al. 2003; Haycraft et al. 2005; Huangfu and Anderson 2005; Liu et al. 2005; May et al. 2005) (detailed in Chap. 2). Ciliary mutant mice have phenotypes similar to those of mutant mice defective in Hedgehog signaling. Epistasis studies placed primary cilia downstream of Hedgehog receptor Patched1 (Ptch1) and Smoothened (Smo), but upstream of the GLI-Kruppel family transcription factors (Gli1-3) and their binding protein Suppressor of Fused (Sufu) (Huangfu et al. 2003; Haycraft et al.

2005; Huangfu and Anderson 2005; Liu et al. 2005; May et al. 2005; Han et al. 2008; Chen et al. 2009; Jia et al. 2009). In the absence of Hedgehog, Ptch1 localizes to primary cilia and inhibits Hedgehog signaling by preventing Smo from entering primary cilia (Rohatgi et al. 2007). Upon binding of Hedgehog, Ptch1 moves out of the cilia, leading to accumulation and activation of Smo in the primary cilium (Corbit et al. 2005; Rohatgi et al. 2007). Activated Smo induces Sufu-Gli complex accumulation in primary cilia and dissociation of the complex, leading to formation of Gli transcriptional activators (Humke et al. 2010; Tukachinsky et al. 2010; Zeng et al. 2010). Without activated Smo in primary cilia, Gli2 and Gli3 are truncated by the proteasome to become transcriptional repressors and repress Hedgehog target gene expression. In the absence of primary cilia, the processing of Gli3 (and probably Gli2 also) repressor is greatly reduced, resulting in derepression of Hedgehog target genes (Haycraft et al. 2005; Huangfu and Anderson 2005; Liu et al. 2005; May et al. 2005).

In contrast to complete loss of primary cilia, which results in complete irresponsiveness to Hedgehog and inefficient processing of Gli repressors, distinctive structural abnormalities in primary cilia cause a range of Hedgehog signaling defects. Mutations in components of intraflagellar transport (IFT) complex A, which functions in retrograde transport of ciliary components from cilia to the cell body, cause abnormally short and swollen cilia and constitutive activation of Hedgehog signaling (Tran et al. 2008; Cortellino et al. 2009). Loss of Arl13b, a ciliary small GTPase, causes opening of axonemal microtubules and constitutive activation of Gli activators at low levels without affecting Gli3 repressor activity (Caspary et al. 2007). Mutations in Broad-minded, a Rab-GAP-like protein, cause detachment of ciliary membrane from the axoneme and selective loss of responsiveness to high Hedgehog levels (Ko et al. 2010). Thus, primary cilia play complex and active roles in Hedgehog signaling rather than merely concentrating signaling molecules to facilitate their interactions.

8.3.2 *Wnt Signaling*

Secreted protein Wnt binds to Frizzled receptors to trigger the signaling activity of cytoplasmic protein Dishevelled (Dvl), where the signaling diverges into canonical and non-canonical Wnt signaling pathways (Logan and Nusse 2004). In the canonical pathway, activated Dvl leads to accumulation and nuclear localization of β -catenin and subsequent activation of Wnt target genes. The non-canonical pathway is independent of β -catenin and primarily controls cytoskeletons involved in planar cell polarity (PCP) and cell migration. One of the first pieces of evidence linking primary cilia to Wnt signaling came from a study on Inversin, a ciliary protein whose mutation causes cystic kidney diseases and *situs inversus* (Simons et al. 2005). The study showed that, in fish and frogs, Inversin functions as a switch from the canonical to the non-canonical Wnt signaling pathway by targeting Dvl for destruction. Moreover, mutant mice defective in genes mutated in Bardet-Biedl syndrome, a disease linked

to ciliary dysfunction, have phenotypes associated with PCP mutants (Ross et al. 2005). Other studies from kidney, pancreas, cochlea, fish embryos, and cultured cells also showed that defective primary cilia increase canonical Wnt signaling activity and disrupt non-canonical Wnt signaling (Lin et al. 2003; Cano et al. 2004; Gerdes et al. 2007; Corbit et al. 2008; Jonassen et al. 2008; Jones et al. 2008). Notably, however, mutant mice defective for primary cilia do not have obvious developmental phenotypes associated with defective Wnt signaling. Furthermore, two recent studies showed that defective primary cilia do not affect Wnt signaling in fish, mice, and cultured cells and suggested that Wnt signaling requires the basal body rather than primary cilia *per se* (Huang and Schier 2009; Ocbina et al. 2009). Thus, the primary cilium and the basal body appear to have a subtle and cell type-specific roles in Wnt signaling (Wallingford and Mitchell 2011).

8.3.3 RTK Signaling

RTKs are activated by growth factors and initiate a series of signaling cascades, including mitogen-activated protein kinase pathways, phosphatidylinositol 3-kinase pathways, and phospholipase C pathways. The first connection between RTKs and primary cilia came from evidence that platelet-derived growth factor receptor α (PDGFR α) signaling requires primary cilia in NIH3T3 cells and mouse embryonic fibroblasts (MEFs) (Schneider et al. 2005). Serum starvation of confluent cells induced PDGFR α expression and its localization to primary cilia. Subsequent stimulation of cells with PDGF-AA ligand induced phosphorylation of PDGFR α and downstream dual specificity mitogen-activated protein kinase kinase 1/2 (MEK1/2) inside primary cilia, leading to phosphorylation of retinoblastoma-associated (RB) protein, which marks S-phase entry. The activation of PDGFR α in primary cilia was also required for directional migration mediated by Na⁺/H⁺ exchanger NHE1 (Schneider et al. 2009). Importantly, PDGF-AA failed to activate PDGFR α to induce S-phase entry and directional migration in MEFs isolated from hypomorphic *IFT88* mutant mice (*IFT88^{Tg737Rpw}*) defective for ciliogenesis. These observations suggest that PDGF-AA and PDGFR α require primary cilia to transmit signals. Some of the observed defects, however, may be partly due to the low level of PDGFR α in *IFT88^{Tg737Rpw}* MEFs after serum starvation, which dramatically induced a higher PDGFR α level in wild-type but not in *IFT88^{Tg737Rpw}* MEFs (Schneider et al. 2005). Thus, it will be important to determine whether PDGF-AA can activate signaling in cells that lack primary cilia but have PDGFR α at a similar level as wild-type cells. It still remains to be determined whether primary cilia are required for PDGFR α signaling *in vivo*.

Another RTK, insulin-like growth factor 1 receptor (IGF1R) appears to function preferentially in primary cilia in MEF-adipose-like 3T3-L1 cells (Zhu et al. 2009), which can be differentiated into adipocyte after growth arrest at confluence. IGF1R activation by insulin is essential to induce differentiation. Interestingly, in 3T3-L1 cells, insulin activated IGF1R in primary cilia faster than IGF1R outside primary

cilia and induced accumulation of activated downstream signaling molecules, phosphorylated insulin receptor substrate 1 (IRS1) and protein kinase B (Akt), at the basal body. Remarkably, knockdown of *IFT88* or *Kif3a* encoding a subunit of essential ciliogenic Kinesin-II motor disrupted IGF1R signaling and blocked differentiation of 3T3-L1, suggesting that IGF1R requires primary cilia to signal to induce differentiation. Like PDGFR α signaling, it is unknown whether primary cilia are required for IGF1R signaling *in vivo*.

In addition to PDGFR α and IGF1R, epidermal growth factor receptor (EGFR) and the angiopoietin receptors Tie-1 and Tie-2 localize to primary cilia (Ma et al. 2005; Teilmann and Christensen 2005; Danilov et al. 2009; Wu et al. 2009). Furthermore, recent studies showed that primary cilia negatively regulate the activity of mammalian target of rapamycin (mTOR) both *in vitro* and *in vivo* (DiBella et al. 2009; Boehlke et al. 2010; Berbari et al. 2011), whereas mTOR positively regulates the length of primary cilia (Yuan et al. 2012). mTOR is a key signaling molecule that integrates RTK signaling with cellular metabolism, a change in which is one of the hallmarks of cancers. Thus, primary cilia appear to participate in multiple RTK signaling pathways, providing them with a platform to crosstalk and integrate. Future work should investigate whether primary cilia participate in RTK signaling *in vivo* and in the crosstalk of multiple RTK signaling pathways.

8.3.4 Notch Signaling

A recent study showed that Notch signaling requires primary cilia during skin development (Ezratty et al. 2011). Notch is a transmembrane receptor protein that undergoes intramembrane proteolytic cleavage upon binding to its ligands, which are mainly transmembrane proteins as well (Kopan and Ilagan 2009). After cleavage, the Notch intracellular domain (NICD) enters the nucleus and activates transcription of target genes. Removing primary cilia in mouse embryonic skin cells by knockdown of *IFT74* or conditional ablation of either *IFT88* or *Kif3a* resulted in defective epidermal differentiation, a process dependent on Notch signaling. Consistently, expression of Notch responsive genes was disrupted in cells lacking primary cilia. Expression of NICD partially rescued expression of a Notch reporter gene and differentiation defects. The study also showed that Notch3 is selectively localized to primary cilia, and Presenilin-2, the catalytic subunit of γ -secretase that cleaves Notch receptor to generate NICD, is localized at the base of primary cilia in addition to intercellular membrane borders. These localizations were specific to suprabasal cells in the embryonic skin, where Notch signaling is active. Remarkably, nuclear NICD3, the processed Notch3, was observed only in ciliated suprabasal cells and not in *Kif3a* mutant cells. These findings raise interesting questions: Does Notch3 require primary cilia to signal in other tissues and animals? Does any other Notch require primary cilia to signal? Are membrane-bound ligands for Notch3 also exclusively localized to a specific domain of the signaling cell to be juxtaposed to the primary cilium of responding cells in developing skin? If so, what is the underlying mechanism?

Why do vertebrate cells require primary cilia for multiple signaling pathways? Simplistically, the concentration of signaling molecules in primary cilia whose large surface area relative to the small volume would provide high sensitivity for detection of low levels of extracellular signals. Subtle structural defects in primary cilia, however, cause unique Hh signaling defects, suggesting that the function of primary cilia are more than concentrating and sensitizing signaling molecules for extracellular signals. The primary cilium, although continuous from cytoplasm, is a distinct subcellular compartment, in which trafficking is restricted by IFT and the transition zone, a barrier at the base of cilia. This restriction may allow the coordinated spread or movement of second messengers or effector molecules. In addition, the juxtaposition of the basal body and the Golgi complex at the base of cilia may facilitate rapid trafficking of molecules like Smo into the cilia upon receiving extracellular signals and could help coordinate the cell cycle.

8.4 Primary Cilia and Medulloblastoma

One of the first bits of direct evidence showing that primary cilia play salient roles in cancer came from a study on medulloblastoma (Han et al. 2009). Medulloblastoma is the most common malignant brain tumor in children, accounting for ~20% of childhood brain tumors. Medulloblastomas mostly arise in the cerebellum, but a recent study showed that a subgroup of medulloblastoma arises in the dorsal brain stem (Gibson et al. 2010). Several transcriptional profiling studies revealed that medulloblastoma comprises four principal subgroups, which have distinct demographic, clinical, transcriptional, and mutational characteristics (Thompson et al. 2006; Kool et al. 2008; Cho et al. 2011; Northcott et al. 2011; Taylor et al. 2012). These subgroups include Sonic Hedgehog (SHH, one of three mammalian Hedgehog proteins), WNT, subgroup 3, and subgroup 4. The SHH and WNT subgroups are named after the signaling pathways thought to drive tumorigenesis of that subgroup. Subgroup 3 often shows amplification of MYC. Molecular mechanisms that drive subgroup 4 have not been identified.

SHH subgroup medulloblastoma is characterized by aberrant activation of SHH signaling and constitutes about 25% of medulloblastoma cases. The SHH subgroup arises from granule neuron precursors (GNPs) in the cerebellum (Schuller et al. 2008; Yang et al. 2008). GNPs are produced from radial glia in the anterior roof of the fourth ventricle, known as the upper rhombic lip, and migrate rostrally to form the external granular layer (EGL) on the surface of the developing cerebellum (Altman and Bayer 1997). In the EGL, GNPs proliferate extensively to produce cerebellar granule neurons, the most abundant neurons that constitute more than half of the neurons in the central nervous system. Immature granule neurons produced from the EGL migrate inward, passing Purkinje neurons and forming the internal granule neuron layer. SHH secreted from Purkinje neurons is an essential mitogen for GNPs in the EGL (Dahmane and Ruiz i Altaba 1999; Wallace 1999; Wechsler-Reya and Scott 1999). Consistent with the critical role of primary cilia in

Hedgehog signaling, GNPs lacking primary cilia failed to proliferate, resulting in severe hypoplasia and underdevelopment of the cerebellum (Chizhikov et al. 2007; Spassky et al. 2008).

While SHH signaling is essential for the proliferation of GNPs, abnormal activation of SHH signaling leads to uncontrolled expansion of GNPs, resulting in medulloblastoma (Hatten and Roussel 2011). A recent study revealed surprising dual roles of primary cilia in medulloblastoma development driven by abnormal activation of SHH signaling (Han et al. 2009). Mice expressing a constitutively active form of Smo (SmoM2), which was identified in medulloblastoma and basal cell carcinoma (Lam et al. 1999), in GNPs develop medulloblastoma (Hallahan et al. 2004; Mao et al. 2006; Schuller et al. 2008; Han et al. 2009). In these mice, SmoM2 concentrated in the primary cilia of tumor cells and required this organelle to induce tumors; concomitant removal of primary cilia in SmoM2-expressing cells completely blocked medulloblastoma development (Han et al. 2009). Unlike SmoM2, GNPs expressing a constitutively active form of GLI2, a downstream transcription factor, that lacks an N-terminal repressor domain (GLI2 Δ N) did not form medulloblastoma (Roessler et al. 2005; Pasca di Magliano et al. 2006; Han et al. 2009). Surprisingly, however, concomitant removal of primary cilia in GNPs expressing GLI2 Δ N resulted in 100% medulloblastoma development, suggesting that primary cilia suppress medulloblastoma development when the oncogenic mutation is in the GLI2 transcription factor. Thus, the primary cilium plays opposing dual roles in medulloblastoma: it is required for SmoM2 but suppressive for GLI2 Δ N to induce medulloblastoma (Fig. 8.1). The molecular mechanism by which primary cilia suppress GLI2 Δ N-driven medulloblastoma development remains to be determined. In the presence of primary cilia, Gli3 repressors may counteract GLI2 Δ N and inhibit medulloblastoma development, whereas in the absence of primary cilia, Gli3 repressors do not form (Haycraft et al. 2005; Huangfu and Anderson 2005; Liu et al. 2005; May et al. 2005), which may allow GLI2 Δ N to induce medulloblastoma. Alternatively, primary cilia may be required for another signaling pathway to suppress tumorigenesis. Primary cilia may also function as a general barrier for cell cycle entry, as discussed above. Similar opposing dual functions of primary cilia were observed in basal cell carcinoma driven by SmoM2 and GLI2 Δ N (Wong et al. 2009). Taken together, these suggest that, to induce cancer, some oncogenic mutations may require intact primary cilia but others may require losing them. Notably, in support of this hypothesis, the presence or absence of primary cilia is tightly associated with specific subgroups of medulloblastoma (Fig. 8.2). In humans, primary cilia are almost exclusively present in the SHH and WNT subgroups of medulloblastoma but absent in subgroups 3 and 4 (Han et al. 2009). Thus, primary cilia may be required for the SHH and WNT subgroups but suppressive for subgroups 3 and 4. The presence of primary cilia in the WNT subgroup is somewhat contradictory to several studies showing that primary cilia constrain canonical WNT signaling; however, as discussed above, the role of primary cilia in WNT signaling is still controversial and specific to cell type. Recently developed mouse models each representing WNT-subgroup medulloblastoma (Gibson et al. 2010) and subgroup 3 (Kawauchi et al. 2012; Pei et al. 2012) will provide an excellent opportunity

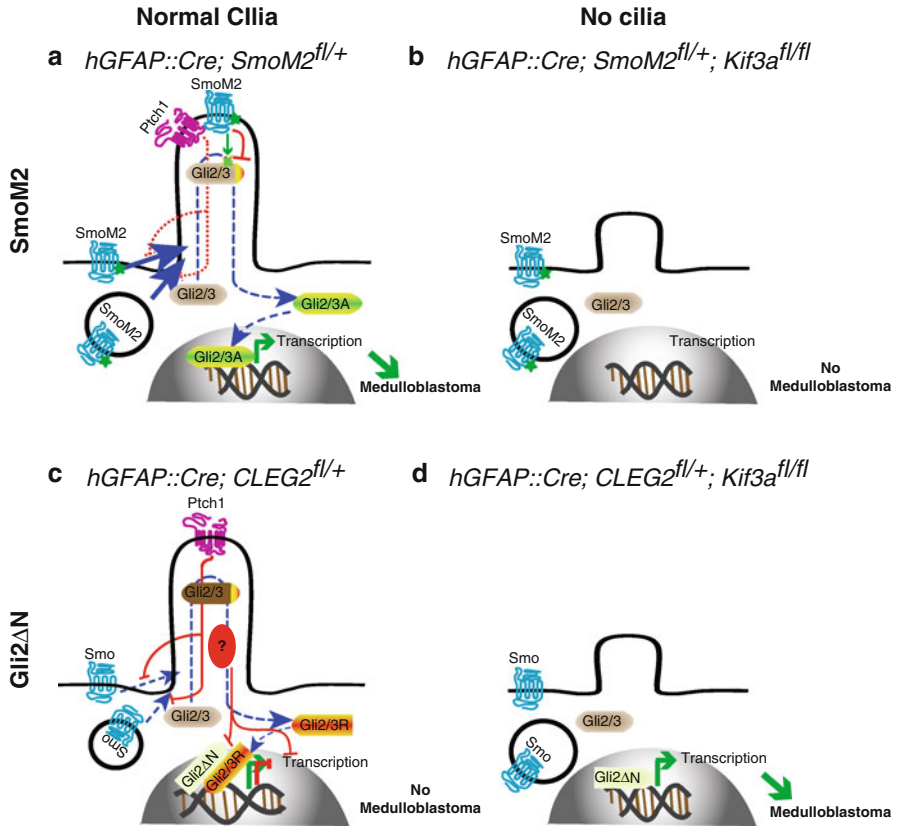


Fig. 8.1 Dual and opposing roles of primary cilia in medulloblastoma formation. (a) SmoM2 is insensitive to inhibition by Ptch1 and constitutively localizes to primary cilia, where it inhibits production of repressor forms of Gli2 and Gli3 and induces production of activator forms. Uncontrolled activation of the signaling leads to medulloblastoma formation in *hGFAP::Cre; SmoM2^{fl/+}* mice. (b) Without primary cilia, SmoM2 cannot activate downstream signaling, thus loss of primary cilia in *hGFAP::Cre; SmoM2^{fl/+}; Kif3a^{fl/fl}* mice completely blocks medulloblastoma formation. (c, d) Constitutively active form of Gli2 (Gli2ΔN) is not sufficient to induce medulloblastoma in *hGFAP::Cre; CLEG2^{fl/+}* mice. Loss of primary cilia in *hGFAP::Cre; CLEG2^{fl/+}; Kif3a^{fl/fl}* mice allows Gli2ΔN to induce medulloblastoma. Repressor forms of Gli2 and/or Gli3, whose formation requires primary cilia, may inhibit the tumorigenic activity of Gli2ΔN. Alternatively, unknown mechanism through primary cilia may inhibit the tumorigenic activity of Gli2ΔN

to investigate the role of primary cilia in these tumors. These investigations will also provide clues to whether the dual roles of primary cilia are generally applicable to cancers in addition to those driven by Hedgehog signaling.

Currently, SMO inhibitors are under clinical trials to treat SHH subgroup medulloblastoma and basal cell carcinoma. Although these clinical trials show promise for SMO inhibitors for treating medulloblastoma, resistance to a SMO inhibitor was observed in a patient who initially showed a dramatic response (Yauch

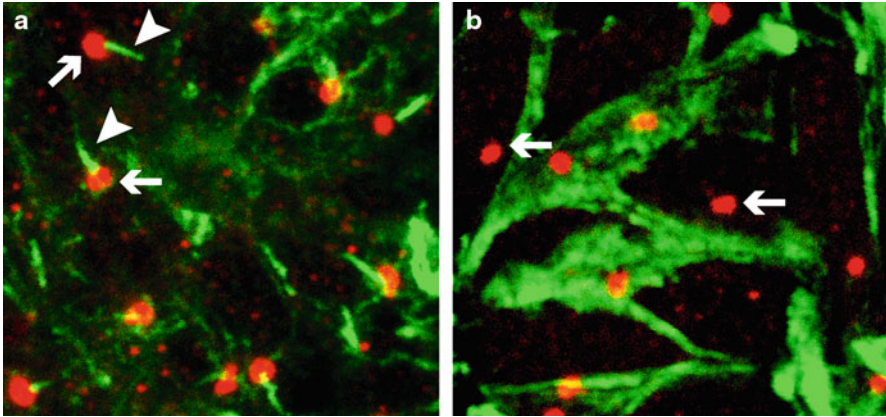


Fig. 8.2 Primary cilia are present in human medulloblastomas showing oncogenic activation of SHH or WNT signaling (a), but mostly absent in other molecular subgroups of medulloblastomas (b). *Arrows* indicate basal bodies stained with an antibody against pericentrin and *arrowheads* indicate primary cilia stained with an antibody against acetylated tubulin

et al. 2009). Subsequently, a point mutation in SMO conferring resistance to the SMO inhibitor was found in the medulloblastoma of this patient. Furthermore, *in vivo* and *in vitro* studies using the same SMO inhibitor revealed frequent appearance of resistance (Dijkgraaf et al. 2011). Currently, SMO is the only molecular target under clinical trials to treat medulloblastoma. Thus, it is necessary to develop strategies to overcome the resistance. Since Smo requires primary cilia to function and ciliogenesis requires a number of cellular processes involving a large number of proteins, primary cilia may provide multiple novel targets that can overcome resistance to SMO inhibitors.

8.5 Primary Cilia and Other Brain Cancers

Given that primary cilia play important roles in cell cycle progression and signaling pathways frequently involved in oncogenesis, they are also likely to play important roles in other brain cancers in addition to medulloblastoma. Here we will discuss two types of brain cancers, the deadliest brain cancers of children and adults, in which primary cilia may have important roles.

Diffuse intrinsic pontine gliomas (DIPGs) are diffusely infiltrative high-grade gliomas in the ventral pons. DIPGs affect mostly children, peaking at age 6–7 years, constituting 10–15% of pediatric brain cancer (Hawkins et al. 2011). DIPGs are extremely aggressive cancers that are almost universally fatal in less than a year. Yet, little is known about the biology of this tumor for which no effective therapy exists. The age- and region-specific natures of DIPGs suggest that these tumors arise from deregulation of a specific postnatal development process occurring in the ventral

pons in children. Indeed, a recent study identified a putative neural precursor cell population positive for neural precursor markers, Nestin, Vimentin, and Olig2 in the ventral pons of both humans and mice, whose spatiotemporal distribution matches very closely to that of DIPGs (Monje et al. 2011). Interestingly, the Hedgehog signaling pathway was active in this precursor population in mice. Expression of SmoM2 increased proliferation of these cells in mice, leading to hypertrophy of the ventral pons. Furthermore, blocking Hedgehog signaling reduced self-renewal of neurospheres generated from human DIPGs, whereas addition of SHH increased self-renewal. Thus, aberrant activation of Hedgehog signaling may contribute to DIPG formation by driving proliferation of these precursor cells. Unlike medulloblastoma, however, expression of SmoM2 was not sufficient to induce DIPGs, suggesting that another hit is necessary for DIPGs to form. This is consistent with the fact that people having germline mutations of PTCH1 are predisposed to medulloblastoma but not DIPG (Johnson et al. 1996). Notably, recent genome-wide analyses identified frequent amplification or overexpression of *PDGFR α* , *PDGF-A*, and *IGF1R*, all of which encode proteins that may signal through primary cilia (Zarghooni et al. 2010; Paugh et al. 2011). Cancer cells that have amplification or overexpression of these oncogenes may rely on normal signaling mechanisms through primary cilia. Thus, primary cilia may play important roles in the development of this devastating disease. It would be interesting to find whether DIPGs are ciliated and co-activation of SHH and *PDGFR α* can cause DIPGs in mouse models, as well as to test the role of primary cilia in such mouse models. If primary cilia play a significant role, targeting ciliogenesis would be a valid treatment option.

Glioma is the most frequent brain tumor in adults, and malignant glioma (glioblastoma multiforme, GBM) comprises 80% of malignant tumors in the central nervous system (Chen et al. 2012). Currently, GBM patients' 5 year survival rate is less than 5%, and median survival is about 1 year. Two recent genome-wide studies including gene expression profiling, DNA copy number variation, protein-encoding gene sequencing, and DNA methylation status revealed three core pathways that commonly mutated in GBM: the p53 pathway, the RB pathway, and the RTK pathway (Parsons et al. 2008; TCGA 2008). The majority (74%) of GBMs had alterations in all three pathways, which enables cancer cells to proliferate unrestrictedly, escaping from cell-cycle checkpoints, senescence, and apoptosis. On the other hand, alterations affecting components in the same core pathway were mutually exclusive. Among RTKs, frequent aberrations were found in EGFR, ERBB2, *PDGFR α* , and MET. Similar to what was seen in medulloblastoma, several gene expression profiling studies identified distinct molecular subtypes in GBM (Vitucci et al. 2011). A recent study grouped GBM into four subtypes: proneural, neural, classical, and mesenchymal (Verhaak et al. 2010). By integrating gene expression profiles with the previous genome-wide analysis of GBM, this study showed that aberrant status of *EGFR*, *NF1*, and *PDGFR α /Isocitrate dehydrogenase 1* define the classic, mesenchymal, and proneural subtypes, respectively. Notably, the classic subtype expressed high levels of Hedgehog (SMO, GLI2, and GAS1) and NOTCH (NOTCH3, JAG1, and LFNG) signaling components. Given the important role of primary cilia in Hedgehog, Notch, and *PDGFR α* signaling pathways, the classic and proneural

subtypes may require primary cilia for their growth. Thus, it would be interesting to determine whether the presence or absence of primary cilia is associated with specific GBM subtypes. Such associations may indicate distinct roles of primary cilia in different GBM subtypes. Primary cilia are absent in several GBM cell lines (Moser et al. 2009); however, the molecular subtypes to which the cell lines belong are unknown. If the presence or absence of primary cilia is associated with specific GBM subgroups and such an association is important for oncogenesis, primary cilia will be an important diagnostic tool and a treatment target for GBM.

Although the cancer stem cell (CSC) theory is controversial, it suggests that primary cilia may have important roles in GBM and possibly in other brain cancers. CSCs are a subpopulation of cells in a cancer that can self-renew and give rise to highly heterogeneous cancer cells that make up the bulk of cancer. GBM CSCs were one of the first CSCs isolated from solid cancers (Singh et al. 2004). Resistance to radiation and chemotherapies is thought to be partly due to CSCs, which have preferentially active DNA repair pathways (Bao et al. 2006) and high levels of ATP-binding cassette transporters to export chemotherapy agents (Bleau et al. 2009); thus, CSCs have important implications for cancer targeting strategy. CSCs are thought to have properties similar to those of somatic stem cells. A number of studies have shown that signaling pathways that critically regulate the behavior of normal somatic stem cells also regulate that of GBM CSCs (Clark et al. 2007; Takebe et al. 2011). These pathways include Hedgehog, Wnt, and Notch signaling pathways, for which primary cilia play important roles. Consistently, recent studies showed that expression of Gli1 and β -catenin are associated with recurrence after therapy and poor prognosis in GBM patients (Rossi et al. 2011; Kim et al. 2012). Therefore, targeting these pathways is a vital therapeutic approach to increase the efficacy of radiation and chemotherapies. Understanding the mechanism by which primary cilia function in these signaling pathways in normal stem cells and CSCs will be important for developing such a therapeutic intervention.

8.6 Conclusion

Brain cancer is a complex and heterogeneous disease. Its treatments, however, are largely similar, including surgical resection, radiation, and chemotherapy, thus resulting in individually different outcomes. Recent advances in genome-wide studies on large cohorts of brain cancer patients elucidated that cancers that otherwise appear identical are highly heterogeneous at the molecular level, with distinctive oncogenic mutations and gene expression profiles. These recent advances call for new treatment paradigms building on better understandings of the molecular and cellular processes involved in initiation and progression of particular brain cancer types. We envision that investigating the function of primary cilia together with oncogenic mutations specific to distinct cancer types will reveal oncogenic mechanisms that were not appreciated previously. We also envision that primary cilia hold a great therapeutic potential for treatment for brain cancer patients. Some cancers

have primary cilia, but others do not (Wheatley 1995; Han et al. 2009; Schraml et al. 2009; Seeley et al. 2009; Wong et al. 2009; Yuan et al. 2010; Kim et al. 2011a). Most cells in our body have the primary cilium; thus, some cancers may have it as a default. Building and maintaining primary cilia requires complex processes involving a wide variety of proteins that function in cell cycle progression (Pugacheva et al. 2007; Spektor et al. 2007; Kim et al. 2011b; Li et al. 2011), cytoskeletal dynamics (Kim et al. 2010a), apicobasal polarity (Fan et al. 2004), planar cell polarity (Kim et al. 2010b; Wallingford 2010), intraflagellar transport (Rosenbaum and Witman 2002), vesicle trafficking (Nachury et al. 2007; Zuo et al. 2009; Knodler et al. 2010), and transcriptional regulation (Thomas et al. 2010). Thus, some cancers may have lost primary cilia secondarily as they progress and accumulate mutations. In cancers driven by Hedgehog signaling, however, the presence or absence of primary cilia directly controls oncogenesis (Han et al. 2009; Wong et al. 2009). Furthermore, the presence or absence of primary cilia is associated with specific cancer types. Thus, the status of primary cilia in a particular cancer may reflect the role of primary cilia in that cancer; some cancers may keep primary cilia and others may eliminate them for growth and progression. Primary cilia will be important targets for such cancers. Since many proteins are involved in ciliogenesis, inhibiting ciliogenesis or ciliary function may be a plausible strategy to treat cancers that require primary cilia for their growth. Indeed, a high-throughput screening for inhibitors of Hedgehog signaling discovered a small molecule that inhibits cytoplasmic dynein and ciliogenesis (Firestone et al. 2012). It will be challenging to restore primary cilia in cancers that have eliminated them for growth. Yet, recent studies showed that small molecules targeting signaling molecules or fatty acid synthesis can restore primary cilia even in cancer cells (Wang et al. 2009; Willemarck et al. 2010). Therefore, research in primary cilia will open up a completely new avenue of research to understand the biology and treatment of cancers.

Acknowledgments American Lebanese Syrian Associated Charities (Y.-G.H.) and the Cancer Center Support Grant CA021765 from the National Cancer Institute (Y.-G.H.) supported this work.

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