Primary Cilium-Mediated Crosstalk of Signaling Cascades in Ciliogenesis: Implications for Tumorigenesis and Senescence

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ABSTRACT: Primary cilium, a small, antenna-like, microtubule (MT)-based extracellular organelle, which extends from the surface of all types of human cells, has important roles in various cellular functions, including planar cell polarity, cell growth, cell cycle, cell migration, transactivation, and immune response. Primary cilium-mediated signaling cascades are initiated by chemosensing environmental signals, such as growth factors and morphogens that activate ciliary receptor-mediated signal transduction, or by mechanosensing of fluid flow followed by induction of intracellular Ca^{2+} flux. Owing to the versatile tools that cilia have, enabling various cellular functions, ciliary dysfunction causes several cilium-related human disorders such as ciliopathies. Here, we focus on the structure and biogenesis of primary cilium and discuss primary cilium-mediated crosstalk of the molecular mechanisms of signaling cascades in ciliogenesis, tumorigenesis, and senescence.

KEYWORDS: primary cilium, ciliopathy, Wnt, Hh, PDGF, tumorigenesis, senescence

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Introduction

The primary cilium is a unique, hair-like, MT-based organelle that projects from the apical surface of vertebrate cells. Most cell types assemble only one cilium (a primary cilium or monocilium), whereas some cells build cilia bundles that consist of 200–300 individual organelles (multiple cilia).[1] The existence of different cilia types indicates that this organelle is likely to have numerous functions. Recent emerging evidence suggests that the primary cilium functions as a sensor for fundamental extracellular factors to transduce signals to the cell body for physiological functions. Most of our knowledge about cilia biology in vertebrate cells is based on genetic studies using *Chlamydomonas reinhardtii* and mouse model systems. Ciliary dysfunction has been shown to cause human disorders, known as *ciliopathies*. Ciliopathies can either involve single organs or present as multisystemic disorders with phenotypically variable and overlapping disease manifestations. In this review, we discuss the structure and biogenesis of primary cilium, typical signal transduction via cilium, and the possible physiological functions of primary cilium in tumorigenesis and senescence that have enabled the development of novel therapeutic options for human ciliopathies.

Structure of Primary Cilium

Initially, primary cilia were considered as solitary cellular appendices without any physiological role that emanated from the surface of most of the quiescent cells in the human body (Fig. 1A). However, through recent accumulating studies, the cilium is believed to include a role as an antenna to receive extracellular signals, in either chemical or mechanical stress pathways, and subsequently mediate these signals to the cell body.[2,3] Primary cilium can be structurally divided into several subcompartments that include a basal body, a transition zone, the cilium (the axoneme and a lipid bilayer ciliary membrane), and the ciliary pocket. Primary cilium harbors nine periphery MT pairs without central MTs—a 9 + 0 axoneme (Fig. 1B). Two types of cilia are known in human beings: 9 + 2 or 9 + 0 MT-based axonemes, and both have been shown to be associated with various human diseases.[2–4] The 9 + 2 axonemes are subdivided into motile cilia (such as respiratory cilia or ependymal cilia) or nonmotile cilia (such as a kinocilium of hair cells of vertebrate inner ears). The 9 + 0 axonemes are subdivided into motile cilia (such as nodal cilia) or nonmotile cilia (primary cilia such as renal monocilia or photoreceptor-connecting cilia; Fig. 1C).[4,5] Primary cilia lack motility because of the

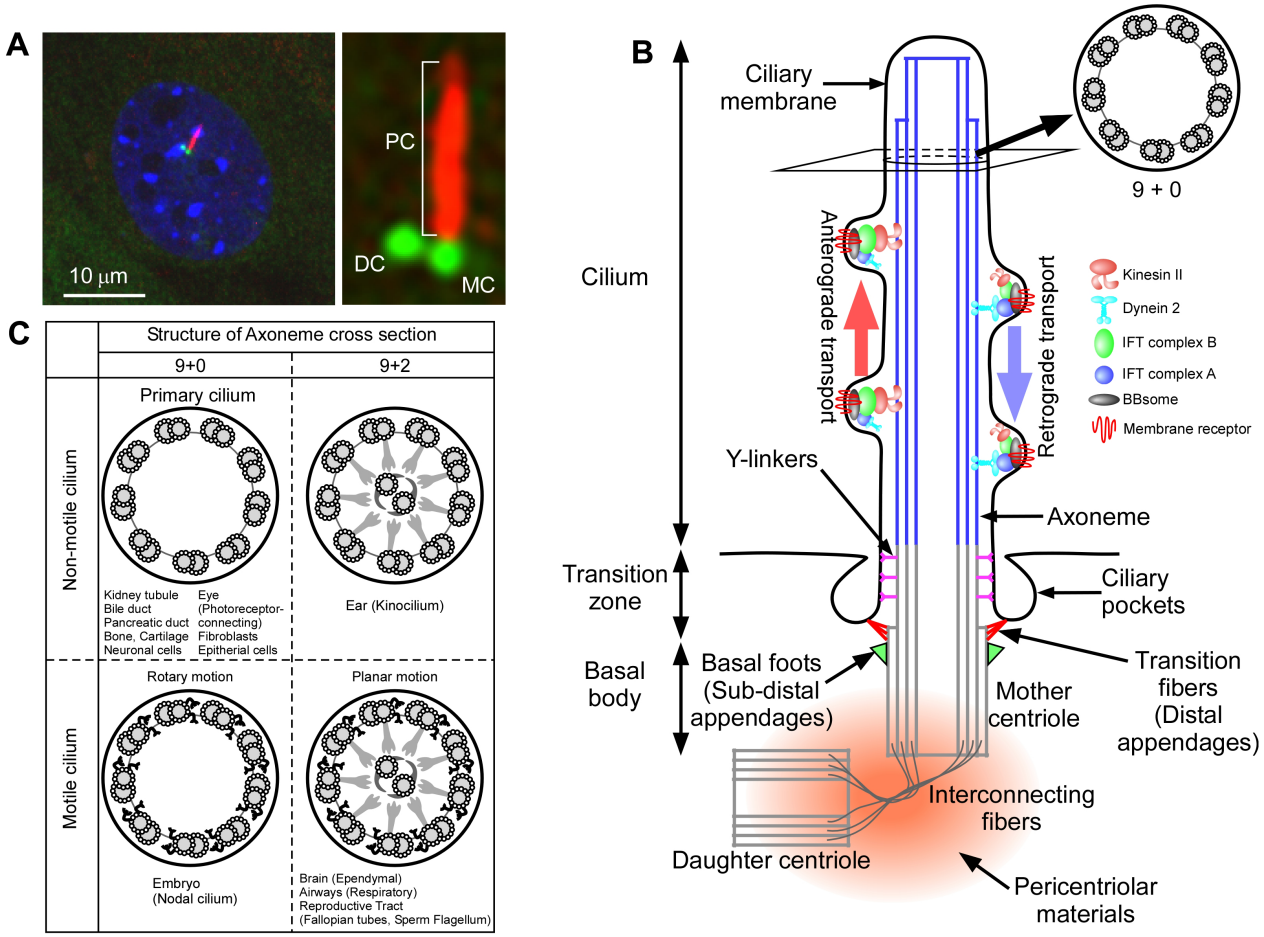

Figure 1. Structure of primary cilium. (**A**) Triple staining of acetylated α-tubulin (red; a marker for the ciliary axoneme), γ-tubulin (green; a marker for either the mother or daughter centriole), and DAPI (blue; a nuclear marker) in 48-hour serum-starved NIH 3T3 mouse fibroblasts. Primary cilium is protruding from the cell surface with the mother centriole as a foothold. PC, primary cilium; MC, mother centriole; DC, daughter centriole. (**B**) Schematic illustration of the primary cilium structure. Primary cilium consists of a ring of a 9 + 0 doublet MT scaffolding called the axoneme, covered by a ciliary membrane. The ciliary transition zone and axoneme arise from the triplet MT-based mother centriole (also named the basal body). The transition zone is characterized by the presence of multiple rows of Y-shaped linkers (Y-linkers) projecting out from the doublet MTs and attaching to the ciliary membrane. At the distal end of the basal body, transition fibers (distal appendages) are anchored to the ciliary membrane to make a ciliary pocket. The basal foot (subdistal appendages) is a structure that polymerizes and anchors the cytoplasmic MT network. Centrosomes are comprised of two centrioles (mother and daughter) connected via interconnecting fibers and pericentriolar materials.[2,131] Kinesin II/IFT complex B-dependent anterograde transport of ciliary components in cilia is important for the assembly processes of cilia. The dynein 2/IFT complex A-dependent retrograde transport system is required for the quality and structural maintenance of cilia and the Hh signal transduction pathway.[15,132] (**C**) Diagram illustrating the structure of axoneme cross sections of cilia, all of which have been associated with human diseases.[2–4] Two types of cilia are known: 9 + 2 or 9 + 0 MT-based axonemes in human beings. The 9 + 2 axonemes are subdivided into motile cilia (such as respiratory cilia or ependymal cilia) or nonmotile cilium (such as a kinocilium of hair cells of vertebrate inner ears). The 9 + 0 axonemes are also subdivided into motile cilia (such as nodal cilia) or nonmotile axonemes (primary cilia such as renal monocilia and photoreceptor-connecting cilia).

absence of the outer and inner dynein arms on the A tubule of the nine doublet MTs, a central pair of MTs, and radial spokes (Fig. 1C). In spite of the structural similarity of all types of cilia, they show diverse, tissue-specific functions during development, tissue morphogenesis, and homeostasis, explaining how cilia-related disorders, such as ciliopathies, can affect many organ systems in the human body. Axonemes of cilia grow with the supply of ciliary components by kinesin/intraflagellar transport (IFT) motor complex-dependent anterograde transport (Fig. 1B). Retrograde dynein/IFT complex-dependent transport is utilized for transducing signaling molecules from cilia to the basal body, which is critical in embryonic and postnatal development and in tissue homeostasis in adulthood.

Assembly of Primary Cilia

The assembly of primary cilia, which is tightly coupled to the cell cycle, is initiated from the distal end of the mother centriole as cells enter the G1/G0 boundary (induced by growth arrest through serum starvation) and is mainly processed by the IFT system (Fig. 2A and B).[6,7] Based on electron microscopy analysis of fibroblasts such as RPE1 (hTERT-immortalized retinal pigment epithelial cell), NIH3T3 (embryonic fibroblast derived from NIH Swiss mouse), or neuronal cells,

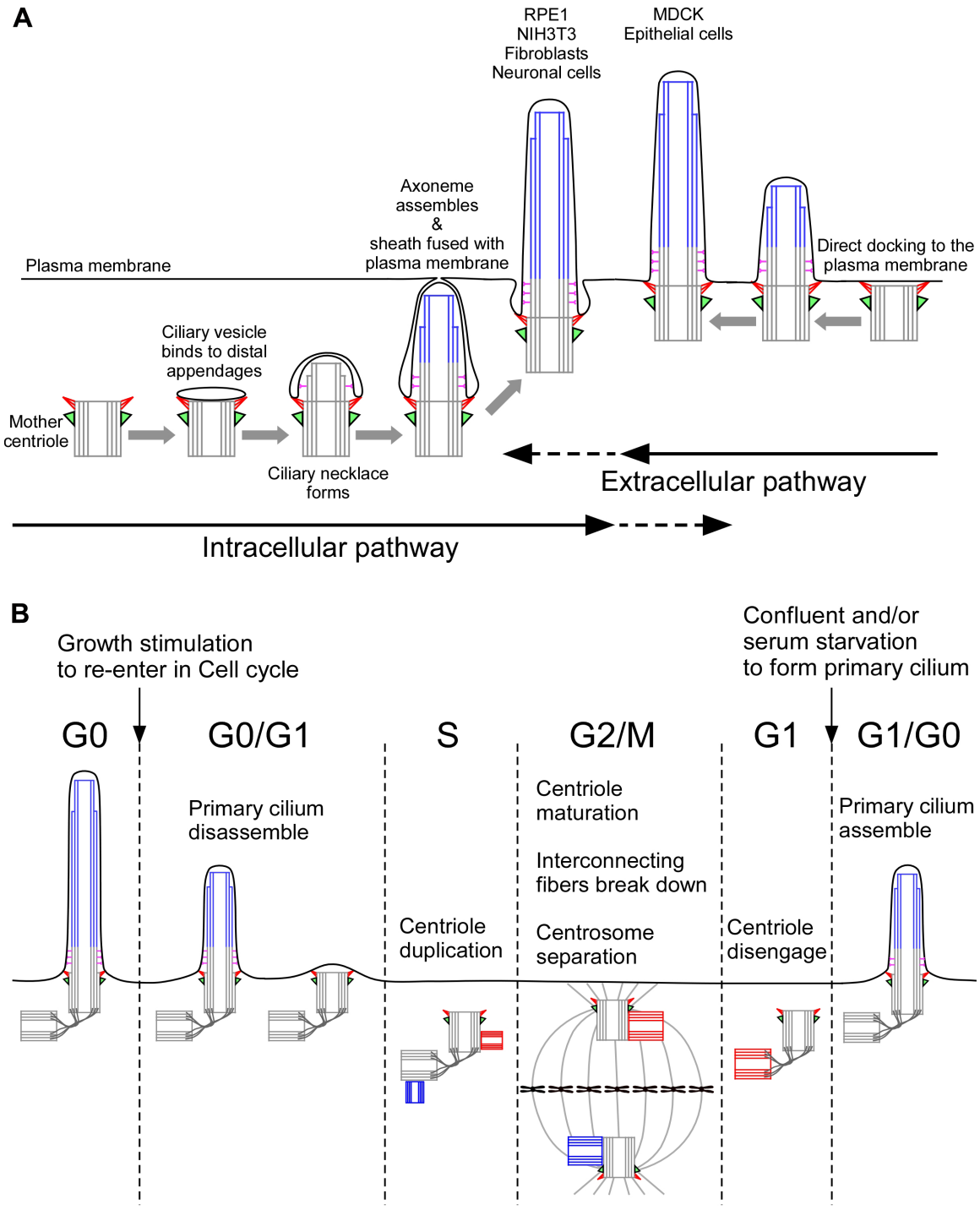

Figure 2. Biogenesis of primary cilium. (**A**) A schematic illustrating intracellular and extracellular ciliogenesis. The intracellular pathway is characterized in human retina RPE1 cells, mouse NIH3T3 cells, other fibroblasts, or neuronal cells. Initially, a ciliary vesicle derived from the Golgi apparatus or endosomes binds to the distal end of the mother centriole via transition fibers (distal appendages). Consequently, a ciliary necklace including the transition zone grows by elongating the axoneme from the mother centriole with Y-linkers. Finally, further axoneme growth, docking, and fusing of the ciliary sheath to the plasma membrane, and subsequent axoneme outgrowth occur to complete ciliary assembly.[133] The extracellular pathway is characterized in Madin–Darby canine kidney cells, the mouse inner medullar collecting duct 3 cell line, and other epithelial cells. In this pathway, axoneme growth begins after direct binding of the mother centriole to the plasma membrane. (**B**) A schematic illustrating cell cycle-dependent ciliogenesis. Primary cilium forms usually in the quiescent stage of the cell cycle. The growth stimulation by various extracellular growth factors renders cell reentry into the cell cycle. Before entering into the cell cycle, primary cilium is disassembled in the G0/G1 boundary. During S phase, centrioles are duplicated to make a copy of the centrosome. During the G2 phase, daughter centrioles are matured and centrosomes are separated by a combination of interconnecting fiber break down, in a CDK1–Aur A–Plk1–Nek2A signaling cascade-dependent manner, and the inactivation of the interconnecting fiber-stabilizing molecule PP1c by CDK1.[134,135] Finally, bipolar spindle poles are formed by separated centrosomes for chromatin segregation in the mitotic phase. During the G1 phase, the mother centriole and duplicated centrioles are disengaged. In order to form primary cilium, cells must be confluent and/or serum starved for at least several hours.

a Golgi-derived ciliary vesicle initially attaches to the distal end of the mother centriole via distal appendages. Subsequently, the surrounding vesicles fuse to the ciliary membrane forming a MT-based ciliary necklace with Y-shaped linkers, which are accessory structures that attach to the ciliary shaft membrane. Finally, the ciliary membrane (sheath)-surrounded axoneme is achieved and fuses to the plasma membrane. The assembly of the primary cilium is thought to be initiated while the mother centriole is positioned at or near the Golgi apparatus and close to the nucleus, prior to centriole migration and the docking of the ciliary sheath to the plasma membrane (Fig. 2A).[8] However, in the case of epithelial cells, such as Madin–Darby canine kidney cells and mouse inner medullary collecting duct cells, the basal body is shown to be translocated and directly attached to the apical plasma membrane, and subsequent elongation of primary cilium takes place. In both situations, the length of the primary cilia can be increased by a considerable size (ranging from 2 to 9 μm).[9]

On the molecular basis of ciliary assembly, the importance of the distal appendages of the mother centriole has been demonstrated by analyzing Odf2 knockout mouse F9 cells, in which the cells are missing the distal appendages (Fig. 1B) together with a failure of primary cilia formation.[10] Functional analyses of many IFT particle proteins, involved in ciliogenesis, have been performed in *Chlamydomonas* and other organisms, including vertebrates. The complete loss of any IFT complex B polypeptides (IFT20, IFT27, IFT46, IFT52, IFT57, IFT80, IFT81, IFT88, and IFT172; Fig. 1B) generally leads to severely shortened or absence of cilia in almost all studied organisms.[11–25] In addition, a number of centrosome proteins (pericentrin, centrin 2, PCM-1, and the Alström syndrome protein, Alms1) were shown to be required for the assembly of primary cilia.[26,27] The anterograde IFT motor kinesin-2 family is also important for ciliary assembly. In a *Chlamydomonas*-based study, a null mutant of FLA10, a subunit of the heterotrimeric-kinesin-2 (kinesin-II) dimer, fails to assemble flagella, indicating that kinesin-II is essential for flagellar assembly in this organism.[28] Consistent with this evidence from *Chlamydomonas*, kinesin-II (a kinesin superfamily protein (KIF)3B or *KIF3A* complex in human beings and mice) is shown to be essential in the assembly of primary cilia using genetic studies in mice; null mutations of either *KIF3B* or *KIF3A* genes lack nodal cilia in embryos and develop situs inversus.[29–31] Analogously, the importance of the retrograde motor cytoplasmic dynein 2 (an isoform of cytoplasmic dynein) of IFT for ciliogenesis and maintenance of cilia is demonstrated by genetic studies using Dync2li1 (a heavy chain of dynein 2)-null mutant mouse embryos. Dync2li1$^{-/-}$ mice fail to assemble full-length cilia and exhibit phenotypes that are consistent with ciliary dysfunction, including embryonic lethality.[32] It is noteworthy that two proteolysis systems are shown to be involved in ciliogenesis. Autophagy, an intracellular degradation system that delivers cytoplasmic constituents to the lysosome, positively regulates assembly of cilium by degrading a ciliary protein, ODF1 (a molecule responsible for an X-linked ciliopathy: oral-facial-digital syndrome 1), which controls the length and distal structure of centrioles.[33,34] In the ubiquitin–proteasome pathways, CRL3-KCTD17 E3 ligase complex-mediated proteolysis of trichoplein initiates axoneme extension during ciliogenesis.[35] A ciliary protein that is encoded by *RPGRIP1L*, a proto-ciliopathy gene, interacts with Psmd2 (a component of the regulatory proteasome 19S subunit) and hence plays an important role in elongation and maintenance of cilium by regulating ciliary proteasome activity.[36] The protein pVHL, which is encoded by the von Hippel–Lindau (VHL) tumor suppressor gene and is a part of an E3 ubiquitin ligase complex, is also involved in ciliogenesis.[37–40] These findings together suggest that quality control and turnover of ciliary components via autophagy and ubiquitin–proteasome pathways play an important role in ciliogenesis, signal transduction via primary cilia, and homeostasis.[35–41]

Disassembly of Primary Cilia

The role of primary cilia disassembly (during G0/G1 boundary in Fig. 2B) on cell cycle progression is still under debate; however, several studies support the idea that primary cilia exert influence on cell cycle progression. For example, Aurora A kinase (AurA)[42,43] and polo-like kinase 1 (PLK1)[44,45] play pivotal roles in growth signal-dependent primary cilia disassembly.[44–47] AurA or PLK1 phosphorylates histone deacetylase 6 (HDAC6) and Kif2A to promote tubulin deacetylation that destabilizes axonemal MTs or depolymerizes the MT of primary cilia, respectively.[44,45] Hef1-dependent phosphorylation of HDAC6 by AurA activation was also reported.[48] Moreover, PLK1 is shown to interact with the Wnt signaling component Disheveled 2 (DVL2) to initiate primary cilia disassembly through the activation of HDAC6, following noncanonical WNT5a ligand stimulation.[46] It has also been reported that Cep97 and CP110, centrosome-implicated proteins, collaborate to inhibit a ciliogenesis program.[49] The levels of ubiquitinated ciliary and flagellar proteins are shown to be increased during flagellar resorption, especially in IFT mutants, suggesting the notion that disassembly products are labeled with ubiquitin and transported to the cell body by IFT.[50]

Ciliopathies

A *ciliopathy* is a human disorder that is directly caused by ciliary malformation or dysfunction due to mutations in proteins that are localized to cilia and/or their basal body.[3] Ciliopathies can manifest kidney and liver defects (including cysts), obesity, and/or mental retardation by central nervous system (CNS) defects. Ciliopathies are also known to exhibit patterning abnormalities in limb length, digit number (polydactyly), left–right axis (situs inversus), craniofacial patterning, and the retina (leading to blindness through defective photoreceptor-connecting cilium). Clinical manifestation of ciliopathies also includes

nephronophthisis, Senior–Løken syndrome, Joubert syndrome, Bardet–Biedl syndrome (BBS), Meckel–Gruber syndrome, and orofacial digital syndrome (OFD),[51,52] for which the responsible genes have been identified.[3,53,54] The mutations of these genes exhibit abnormal cilia in terms of length (short or absent), shape (bulged), and density (sparse) together with Hedgehog (Hh) signaling defects. Therefore, pivotal mechanisms of clinical features in ciliopathies are most likely due to abnormal Hh signaling through primary cilia. Alternatively, dysfunction of motile cilia is known as primary ciliary dyskinesia, which appears in cells and tissues of various human diseases. Poor mucociliary clearance caused by dysfunctional airway cilia leads to chronic infections, sinusitis, and rhinitis, which can result in the widening of the airways and lung collapse (bronchiectasis and atelectasis, respectively). It has been reported that the lack of motility of sperm flagella and motile cilia in the oviducts can lead to infertility, whereas dysmotility of cilia in the embryonic node leads to left–right patterning abnormality (situs inversus, also known as Kartagener's syndrome). In some rare cases, defects in ependymal motile cilia of the CNS are shown to manifest in the swelling of brain ventricles or hydrocephalus.[2,54–56] The molecular defects in trafficking of ciliary G protein-coupled receptors, such as melanin-concentrating hormone receptor 1 and somatostatin receptor subtype 3 to neuronal cilia, resulted in obesity by deregulation of the feeding and energy balance, and blindness by retinal degeneration.[57] In addition, patients with BBS often manifest obesity and cognitive impairments due to neuronal defects; however, it is of note that the specific pathways responsible for these attributes in patients with BBS have not been identified clearly.[3]

Chemosensation-based Signaling Cascade

Hh signaling. Cloning the secreted proteins of the Hh family was initiated two decades ago, and the field of Hh study has diversified into encompassing embryonic development, stem cell biology, and tissue homeostasis (Fig. 3A). The focus of current Hh signaling pathway studies is determining whether the pathway is implicated in several cancers and congenital syndromes.[58] In the off-state (absence of Hh), the Hh receptor Patched-1 (Ptch1) interferes with the localization of smoothened (SMO; an activator of the GLI transcription factor) to primary cilia. In this scenario, suppressor of fused (SUFU)-dependent phosphorylation of GLI by protein kinase A (also known as cyclic AMP-dependent protein kinase), casein kinase 1 (CK1), or glycogen synthase kinase 3 beta (GSK-3β) initiates partial degradation of GLI via the ubiquitin–proteasome pathway, and the short form of GLI functions as a repressor for the transactivation of the target gene. After Hh stimulation, its receptor, Ptch1, is anchored in the plasma membrane with the Hh molecule and loses the ability to inhibit SMO translocation to cilium. Thus, SMO inhibits SUFU in primary cilia and allows full-length GLI to translocate from cilia to the cytoplasm. Consequently, full-length GLI can function as an activator for the transactivation of target genes in the nucleus.[59–61] Numerous genetic evidence in mice supports the idea that Hh signaling is strictly mediated through the primary cilia.[59–62] Consistently, impaired formation or functioning of the primary cilium results in diverse human diseases, including ciliopathies, by Hh signaling defects.[2,3,54–56]

Canonical Wnt signaling. The Wnt signaling pathway is highly conserved evolutionarily in many species, ranging from fruit flies to human beings, and is mediated through the primary cilia machinery (Fig. 3B).[63–65] The Wnt pathway is also shown to control embryonic processes including body axis patterning, cell fate specification, cell proliferation, and cell migration. The clinical importance of the Wnt pathway has been demonstrated by mutations that lead to various diseases, including breast and prostate cancer, glioblastoma, and type II diabetes.[66,67] The Wnt pathways are largely classified into two

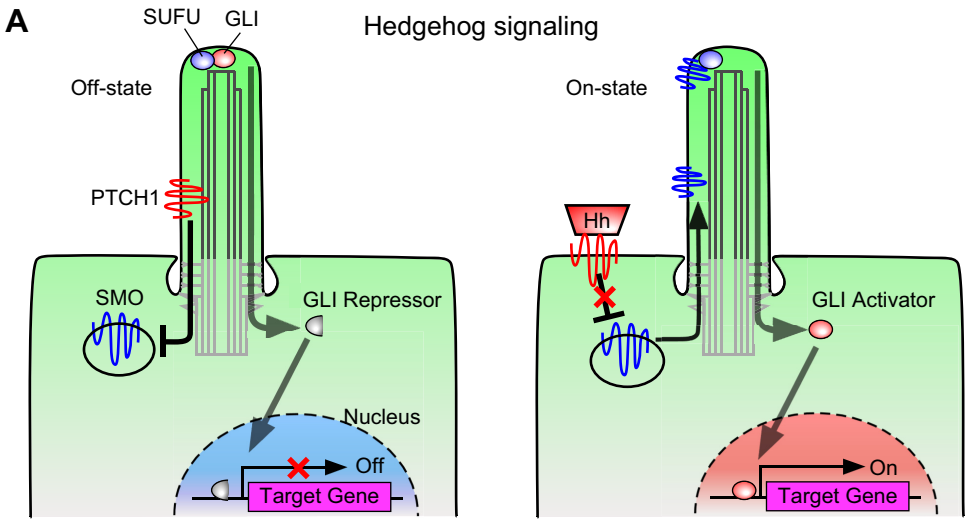

Figure 3. (*Continued*)

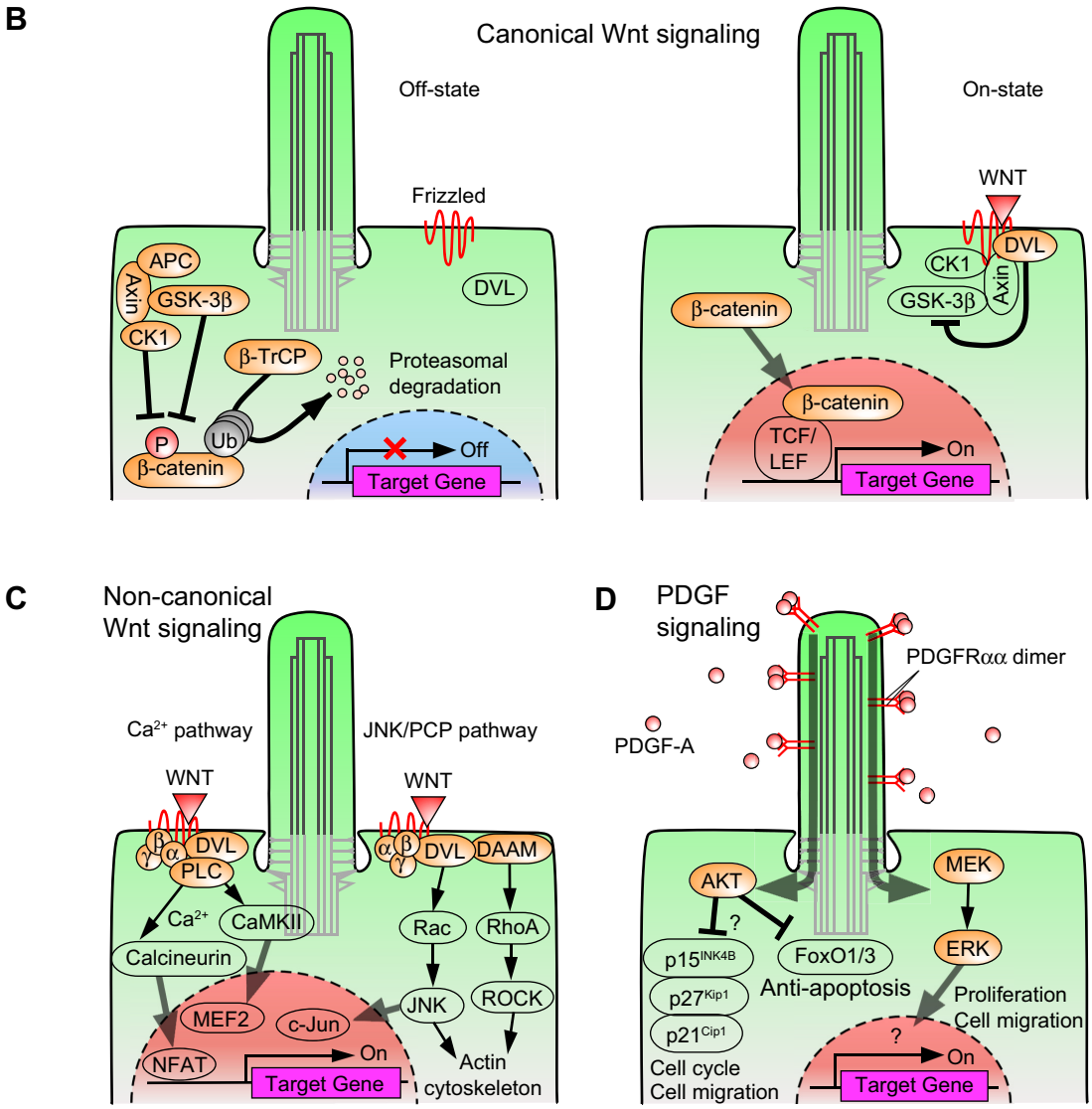

Figure 3. The chemosensation-based signal transduction. (**A**) A schematic illustrating Hh signaling. In the off-state, Ptch1 interferes with the ciliary localization of SMO, which is an activator of the GLI transcription factor. In this situation, the phosphorylated GLI molecules, by protein kinase A (also known as cyclic AMP-dependent protein kinase), CK1, or GSK-3β in a SUFU-dependent manner, are partially degraded by the ubiquitin–proteasome pathway; consequently, partially digested GLI functions as a repressor for the transactivation of the target gene. After Hh stimulation, its receptor, Ptch1, is anchored in the plasma membrane with the Hh molecule and loses its inhibitory activity against the ciliary localization of SMO. SMO moves into cilium to inhibit SUFU, which allows full-length GLI to translocate into the nucleus to function as a GLI activator for the transactivation of the target genes. (**B**) A schematic illustrating canonical Wnt signaling. In the off-state of Wnt signaling, a key transcription factor, β-catenin, is phosphorylated by the destruction complex (Axin, APC, CK1, and GSK-3β) and is consequently ubiquitinated and degraded in the β-TrCP/SCF-dependent ubiquitin–proteasome pathway. DVL is shown to be an important molecule that positively regulates the canonical Wnt signaling pathway by inhibiting GSK-3β activity.[68] In the on state by WNT stimulation, DVL recruits the destruction complex to the plasma membrane and inhibits the ubiquitination activity of the complex against β-catenin. DVL indirectly functions as a positive regulator of Wnt signaling by enabling β-catenin to accumulate in the cytoplasm and translocate into the nucleus. Therefore, β-catenin is suggested to have a role as a transcriptional coactivator with T-cell-specific transcription factor/LEF transcription factor family and induce WNT target genes.[69] (**C**) A schematic illustrating noncanonical Wnt signaling. JNK/PCP pathways (*right*) and Ca^{2+} pathways (*left*) are shown. In both cases, G proteins (α, β, and γ) are activated through WNT binding to the receptor Frizzled and recruit DVL to the membrane to initiate signaling. On the JNK/PCP pathway, activation of small GTPases (RhoA and Rac) is required to activate JNK and Rho-kinase (ROCK) to regulate the rearrangement of the actin-based cytoskeleton and cell polarity. In the Ca^{2+} pathway, both G proteins and DVL activate PLC, a generator of IP_3 and diacylglycerol (DAG). These lipids increase intracellular Ca^{2+} levels and activate CAMKII, calcineurin, protein kinase C, and ERK, eventually leading to activation of transcription factors such as nuclear factor of activated T-cells and myocyte-specific enhancer factor 2.[71] (**D**) A schematic illustrating PDGF signaling. AKT (also known as protein kinase B) and MEK cascades are activated upon PDGF-A-mediated stimulation through the ciliary PDGFRα receptor dimer.[79] It has been reported that AKT inhibits the transactivation of FoxO1/3-dependent apoptosis-related genes for cell survival and activates the CDK4/6/cyclin D and CDK2/cyclin E complexes by inhibiting $p15^{INK4B}$, $p27^{Kip1}$, and $p21^{Cip1}$, allowing cell cycle reentry from the G0/G1 phase.[81,82] MAPK-mediated phosphorylation of the transcription factors, c-Myc and c-Fos, and cytoskeletal proteins, focal adhesion kinase and Paxillin, have been shown to play an important role in the regulation of cell proliferation and migration, respectively.[80,83,136,137]

pathways: β-catenin-dependent *canonical* Wnt signaling or β-catenin-independent *noncanonical* Wnt signaling. In the off-state of the canonical pathway, a key transcription factor, β-catenin, is degraded by the destruction complex [Axin, adenomatous polyposis coli (APC), CK1, and GSK-3β] in the β-TrCP/SCF-dependent ubiquitin–proteasome pathway. Under this circumstance, the phosphorylation of β-catenin by CK1 and GSK-3β is identified as a trigger for degradation. DVL positively regulates canonical Wnt signaling by direct inhibition of GSK-3β kinase activity.[68] On the contrary, in the on-state, the activity of the destruction complex against β-catenin is silenced by its relocation and anchoring to the plasma membrane by DVL. As a consequence, the accumulated cytoplasmic β-catenin migrates into the nucleus to function as a transcriptional coactivator with the T-cell-specific transcription factor/LEF transcription factor family for the induction of WNT target genes.[69]

Noncanonical Wnt signaling. Noncanonical Wnt signaling is thought to be independent of β-catenin, but dependent on DVL (Fig. 3C). The mechanosensation-dependent elimination of both β-catenin and DVL molecules in the Ca^{2+} pathway (shown in Fig. 4) is believed to be the important initiator for noncanonical Wnt signaling. Under urea flow, DVL is degraded in an APC/C E3 ligase/inversin (INVS) complex-dependent manner. At the same time, β-catenin is also degraded due to the absence of DVL as shown in Figure 3B (off-state). In some context, INVS is also shown to be ubiquitinated and degraded through APC/C E3 ligase and the proteasome pathway. Degradation of INVS stabilizes DVL and consequently maintains a high level of DVL expression in the cytoplasm; high levels of DVL trigger the noncanonical signaling cascade in a WNT ligand and G protein-dependent manner (Fig. 3). Two noncanonical Wnt signaling pathways (C-Jun N-terminal kinase (JNK)/planar cell polarity (PCP) pathways (*right*) and Ca^{2+} pathways (*left*)) both require the recruitment of DVL to the membrane through the G protein–Frizzled receptor complex with WNT. Rearrangement of the actin-based cytoskeleton by the JNK and Rho-kinase (ROCK) via small GTPases (RhoA and Rac) is shown to be important for the regulation of PCP.[70] The Ca^{2+} pathways of noncanonical Wnt signaling activate phospholipase C (PLC), a generator of inositol triphosphate (IP_3) and diacylglycerol (DAG). Intracellular Ca^{2+} release by PLC activation upregulates Ca^{2+}/CaM-dependent-dependent kinase II (CAMKII), calcineurin, protein kinase C, and extracellular signal-regulated kinase (ERK). Finally, Ca^{2+}-mediated signal cascades induce transactivation of target genes, mediated by nuclear factor of activated T-cells and myocyte-specific enhancer factor 2.[71]

It has been reported that cilia do not play a role in Wnt signaling during development.[62,72] Three different mutant mice strains (with a mutation in *ift188*, *ift72*, or *kif3a*) have been generated, in which the defects of IFT presumably lack cilia; however, no obvious abnormality in the Wnt-dependent

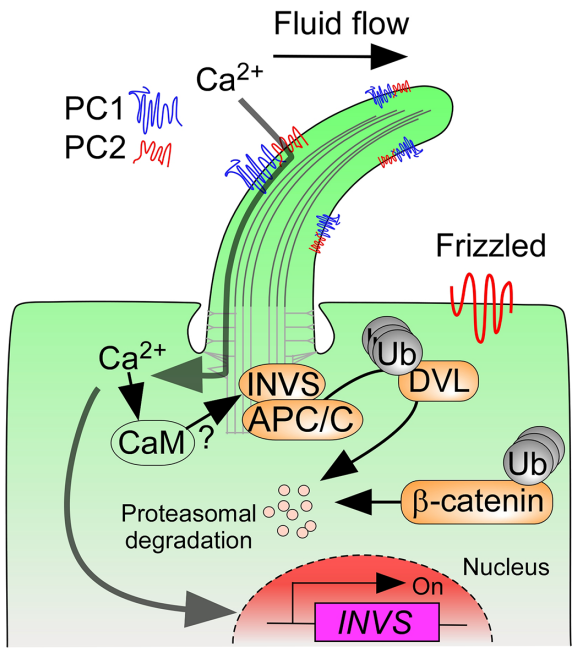

Figure 4. A schematic illustration of the mechanosensation-based Ca^{2+} signaling pathway. Mechanosensation such as urine flow over primary cilia induces intracellular Ca^{2+} flux via the PC1/PC2 ion channel complex. The expression of INVS, a protein originally discovered for its role in situs inversus and cystic renal diseases, which mainly localizes in INVS compartments including the transition zone of the primary cilium, is induced in an increased intracellular Ca^{2+}-dependent manner.[73,97] INVS is shown to play a role as a molecular switch to modulate canonical and noncanonical Wnt signaling by contributing to DVL degradation through the APC/C (an E3 ubiquitin ligase)-mediated ubiquitin–proteasome pathway.[54,73,98,100] Concomitant with DVL degradation, elimination of β-catenin also occurs in a destruction complex-dependent manner (Fig. 3B) because of the absence of DVL, an inhibitor of GSK-3β. Once extracellular WNT molecules bind to the receptor Frizzled, the noncanonical Wnt pathway is initiated in a β-catenin-independent manner by recruiting DVL to the plasma membrane, which enables DVL to avoid APC/C-dependent proteasomal degradation (Fig. 3C).[2,54]

developmental events are observed.[72] Consistently, *ift188* mutant zebrafish zygotes also lack all cilia and show Hh signaling defects; however, canonical and noncanonical Wnt signaling remain intact. It is of note that there is controversy over the evidence reported in the connection between cilia and Wnt signaling. The level of canonical Wnt signaling appeared to be negatively regulated by the presence of primary cilium in cultured cells and zebrafish embryos.[64,65,73–77] These reports supported the notion that primary cilia regulate Wnt signaling by acting as a switch between the canonical and noncanonical states. In this regard, Lancaster et al have shown that Jouberin protein recruits β-catenin to the primary cilium to keep the nuclear level of β-catenin and the amount of Wnt signaling low, indicating that in some context Wnt signaling is actually controlled via primary cilia.[77,78]

Platelet-derived growth factor signaling. Christensen et al reported that platelet-derived growth factor (PDGF) receptor α (PDGFRα) is dominantly localized to a portion

of primary cilia rather than plasma membrane (Fig. 4).[79,80] Upon stimulation by PDGF-A, but not PDGF-B, through the ciliary PDGFRα dimer, AKT (also known as protein kinase B) and mitogen-activated kinase (MAPK)/ERK kinase (MEK) cascades are activated. The molecular function of ciliary-specific AKT activation is not clear; however, it is possible that activation of AKT results in the inhibition of the apoptosis-related transcription factor FoxO1/3 and activation of the cyclin-dependent kinase (CDK)4/6/cyclin D and CDK2/cyclin E complexes by inhibiting $p15^{INK4B}$, $p27^{Kip1}$, and $p21^{Cip1}$, promoting cell survive and cell cycle reentry from the G0/G1 phase.[81,82] MAPK may also participate in the phosphorylation of transcription factors for proliferation and cell migration.[80–83] In addition to the importance of PDGFRα-mediated signaling through the cilia for directed migration in fibroblasts, the receptor is found to localize to primary cilia in vivo, in neural stem cells of the adult rat subventricular zone.[84] Loss of PDGF signaling does not result in severe phenotypes in early mouse embryos; however, it is crucial for tissue development of later stages, including oligodendrocytes and neural crest-derived craniofacial structures.[85,86] Notably, the PDGF signaling pathway is reported to be essential for normal embryogenesis, inflammation, and wound healing. Therefore, unregulated activation of the PDGF signal pathway is associated with numerous disorders, including carcinogenesis. Upon the binding of the ligand, the PDGF receptor dimerization and autophosphorylation trigger the phosphorylation of intracellular signaling molecules to transduce downstream signaling cascades, which include the phosphoinositide 3-kinase (PI3K)/AKT and MEK pathways. Primary cilia of fibroblasts from *Tg737*^{orpk} (*Ift88*^{Tg737Rpw}) mutant mice are structurally abnormal (absent or shortened) and failed to upregulate the MEK1/2-ERK1/2 and PI3K/AKT pathways through PDGFRα upon PDGF-AA stimulation. This observation supported the idea that primary cilia are essential for PDGF signaling and its downstream effectors through PDGFRα.[80] Other molecules, including transforming growth factor-β, fibroblast growth factor, epidermal growth factor, somatostatin receptor subtype 3, leptin, insulin-like growth factor-1, and integrins, have also been reported to mediate signal transduction through the ciliary receptor; these observations support the notion that more complex signaling crosstalk through the primary cilium may be linked to various human diseases.[87–96]

Mechanosensation-Based Ca^{2+} Signaling

Mechanosensation such as urine flow over primary cilia induces intracellular Ca^{2+} flux via the polycystin-1 (PC1)/polycystin-2 (PC2) ion channel complex (Fig. 4). Consequently, the expression of INVS, a protein originally discovered for its role in situs inversus and cystic renal diseases and mainly localized in the INVS compartment including the transition zone of primary cilia, is transcriptionally elevated.[97] INVS is shown to have a role as a molecular switch to modulate canonical and noncanonical Wnt signaling through facilitating the DVL degradation primarily through the anaphase-promoting complex/cyclosome (APC/C, an E3 ubiquitin ligase)-mediated ubiquitin–proteasome pathway.[54,73,98–100] Concomitant with DVL degradation, destruction complex-dependent β-catenin degradation (Fig. 2B) occurs because of the absence of GSK-3β inhibition by DVL, which then keeps cells in the off-state of noncanonical Wnt signaling. Once extracellular WNT molecules bind to the receptor, Frizzled, the noncanonical Wnt pathway is subsequently activated in a β-catenin-independent manner by recruiting DVL to the plasma membrane to prevent the proteasomal degradation of DVL (Fig. 3C).[2,54]

Primary Cilia and Tumorigenesis

The role of primary cilia in cancer has not been entirely characterized to date. However, primary cilia are absent or shortened in several types of cancer, including kidney, skin, brain, breast, pancreas, and prostate.[101–110] It is also reported that a reduction in cilia frequency relative to adjacent normal tissues was observed in renal cell carcinoma (clear cell), breast cancers, melanoma, basal cell carcinoma, medulloblastoma, and pancreatic cancer.[104,105,107,110–119] Genetic studies further support that the loss of cilia can increase tumor incidence in basal cell carcinoma and medulloblastoma.[102–117] It has been reported that primary cilia are lost early in breast cancer development on both the cancer cells and their surrounding stromal cells. However, loss of cilia may not be sufficient to initiate tumorigenesis in the mammary gland.[110] Furthermore, the loss of primary cilia is not associated with positive staining of Ki67 (a cell proliferation marker) in melanoma, renal cell carcinoma, and pancreatic cancer.[104,105,112] Therefore, loss of cilia may not be due to a result of altered cellular proliferation rates; primary cilia dysfunction in cancers may be caused by other mechanisms, such as a loss of genes required for ciliogenesis, potentially resulting from mutagenesis or genomic instability. Although cilia dysfunction is commonly observed in cancers and during an early tumorigenic process, further work is necessary to elucidate the precise relationship of ciliogenesis and tumorigenesis.[112] Primary cilia of human embryonic, neuronal, and cancer stem cells (CSCs) are shown to play a role in the regulation of development, tissue differentiation, and tumorigenesis. Hh signaling is shown to be a main mechanism in this regulation. However, some populations such as CSC medulloblastoma (CD15+ cells) lack primary cilia and are independent of Hh signaling for tumorigenesis.[118] Therefore, further work with additional types of cancer is needed in order to clarify the regulatory molecular mechanisms of CSCs through primary cilia.[112]

Abnormal Hh activity is also involved in various cancers, including basal cell carcinomas, medulloblastomas, rhabdomyosarcomas, glioblastomas, and breast and prostate cancers.[108–110] WNT molecules and their downstream effectors regulate various stages of cancer development including initiation, progression, and metastasis. Furthermore, expression of

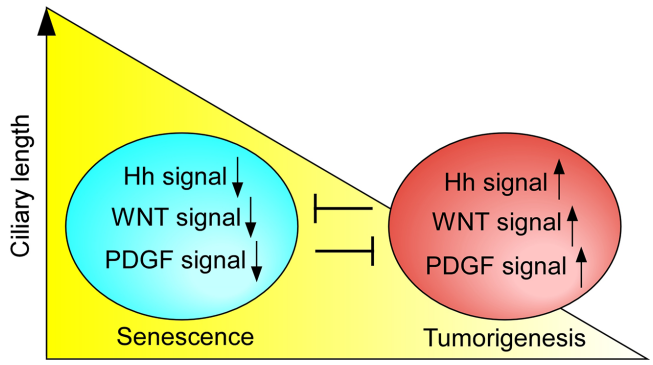

Figure 5. A schematic representing the relationship between ciliogenesis, tumorigenesis, and senescence. Three cellular events, ciliogenesis, tumorigenesis, and senescence, are suggested to interconnect and crosstalk via the chemosensation-mediated signaling cascades, such as Hh, Wnt, and PDGF, through the primary cilia. However, further studies are required to elucidate how these signals indeed crosstalk to maintain cellular homeostasis in vivo.

PDGF and its receptor was found in various tumors including those in the CNS, germ cells, and the gastrointestinal system. PDGF not only facilitates cellular proliferation of tumors but also provides a growth advantage to the adjacent cells and/or angiogenesis.[120] It has been reported that PDGF alone triggers the disassembly of the primary cilium.[121] As mentioned above, Hh signaling is strictly mediated through primary cilia.[59–62] The activity of Wnt signaling is also regulated by primary cilia.[64,65,77] Three signaling pathways through Hh, Wnt, and PDGF are indeed regulated by primary cilia; therefore, the molecules responsible for chemosensation-based signal transduction and ciliogenesis would appear to be therapeutic targets for cancer (Fig. 5). Furthermore, two ciliary proteins encoding tumor suppressors (pVHL, a component of E3 ligase, or serine/threonine kinase 11 (LKB1) kinase) also play an important role in the maintenance of primary cilia and their downstream signaling effects such as cell cycle and cell size regulation.[122–124] Further study of tumor suppressive functions of ciliogenesis may provide a new insight into the molecular mechanisms involved in the mechanisms related to human cancer.

Primary Cilia and Senescence

Normal cells enter a senescent state upon aberrant oncogenic signaling to inhibit tumor initiation and progression, which coincides with the elongation of primary cilia in these cells.[125] In senescence, the proportion of the cell with primary cilia is increased together with transient cell cycle arrest. However, it remains to be addressed whether senescence promotes ciliogenesis and vice versa.[126–128] Breslin et al reported that senescent (late passage) human fibroblasts display both an increased frequency and length of primary cilia, compared to proliferating (young passage) fibroblasts.[126] Importantly, the levels of Hh signaling are decreased in fibroblasts in a replicative senescence stage compared to the normally proliferating cells. Inhibition of Hh signaling both attenuated cell proliferation and the elongation of primary cilia, suggesting that Hh signaling inhibits ciliogenesis and replicative senescence in human fibroblasts. WNT inhibitory factor 1 (WIF1) not only suppresses cancer stemness but also induces cellular senescence. It remains to be elucidated whether ciliogenesis is affected by WIF1 or Wnt signaling itself.[129] However, the combined treatment of PDGF-AA and the PTEN inhibitor bpV(pic) promotes self-renewal in human skin-derived precursors and embryonic neural crest somite-derived multipotent progenitor cells, which alleviated cellular senescence in vitro. The PI3K inhibitor LY294002 is known to block the cellular self-renewal by PDGF-AA and bpV(pic) treatment; therefore, PI3K/AKT signaling plays an important role in the inhibition of cellular senescence progression.[130]

Conclusion and Perspective

Three chemosensation signaling cascades through Hh, Wnt, and PDGF are considered to interconnect and crosstalk for the cellular processes of ciliogenesis, senescence, and/or tumorigenesis. Furthermore, ciliogenesis is thought to be involved in the regulation of senescence and tumorigenesis (Fig. 5). However, the proportion of signaling events (eg, Hh, Wnt, and PDGF) that is solely through primary cilia and not through the nonciliary mediated signaling of the cell surface receptor remains to be elucidated. Moreover, it is difficult to distinguish between cilium-mediated signal transduction and nonciliary cascades in the field of cell biology. Further study is required to clarify whether the signaling cascades and downstream cellular events are solely regulated through primary cilia and how these downstream signaling events crosstalk at a molecular level for the maintenance of the mammalian cellular homeostasis in vivo.

Abbreviations

AKT, v-akt murine thymoma viral oncogene homolog (also known as protein kinase B); APC, adenomatous polyposis coli; APC/C, anaphase-promoting complex/cyclosome; AurA, Aurora A kinase; BBS, Bardet–Biedl syndrome; CAMKII, Ca^{2+}/CaM-dependent-dependent kinase II; CDK1, cyclin-dependent kinase 1; CK1, casein kinase 1; CNS, central nervous system; DAG, diacylglycerol; DVL, disheveled; EGF, epidermal growth factor; ERK, extracellular signal-regulated kinase; FAK, focal adhesion kinase; FGF, fibroblast growth factors; GSK3-β, glycogen synthase kinase 3 beta; HDAC6, histone deacetylase 6; Hh, Hedgehog; IFT, intraflagellar transport; IGF-1, insulin-like growth factor-1; INVS, Inversin; IP3, inositol triphosphate; JBTS, Joubert syndrome; JNK, C-Jun N-terminal kinase; KIF, Kinesin superfamily protein; LKB1, serine/threonine kinase 11; MAPK, mitogen-activated kinase; MCHR1, melanin-concentrating hormone receptor 1; MDCK cells, Madin–Darby canine kidney cells; mIMCD

cells, mouse inner medullary collecting duct cells; MEF2, myocyte-specific enhancer factor 2; MEK, MAPK/ERK kinase; MKS, Meckel–Gruber syndrome; NFAT, nuclear factor of activated T-cells; Nek2A, NIMA (never in mitosis gene a)-related kinase 2A; NIH3T3, embryonic fibroblast derived from NIH Swiss mouse; NPHP, nephronophthisis; LEF, lymphoid enhancer binding factor; OFD, oral-facial-digital syndrome; PC1, polycystin-1; PC2, polycystin-2; PCD, primary ciliary dyskinesia; PCP, planar cell polarity; PDGF-A, platelet-derived growth factor-A; PDGFRα, platelet-derived growth factor receptor α; PI3K, phosphoinositide 3-kinase; PKA, protein kinase A (also known as cyclic AMP-dependent protein kinase); PKC, protein kinase C; PLC, phospholipase C; PLK1, polo-like kinase 1; Ptch1, Patched-1; RPE1 cells, hTERT-immortalized retinal pigment epithelial cell line; ROCK, Rho-kinase; SLS, Senior–Løken syndrome; SMO, smoothened; SSTR3, somatostatin receptor subtype 3; TCF, T-cell-specific transcription factor; TGF-β, transforming growth factor β; VHL, von Hippel–Lindau; WIF1, WNT inhibitory factor 1.

Author Contributions

Conceived the concepts: FS and MN. Analyzed the data: FS, NH, SI, TE, KK, TT, and MN. Wrote the first draft of the manuscript: FS. All authors reviewed and approved of the final manuscript.

REFERENCES

1. Tang TK. Centriole biogenesis in multiciliated cells. *Nat Cell Biol*. 2013;15(12):1400–1402.
2. Fliegauf M, Benzing T, Omran H. When cilia go bad: cilia defects and ciliopathies. *Nat Rev Mol Cell Biol*. 2007;8(11):880–893.
3. Goetz SC, Anderson KV. The primary cilium: a signalling centre during vertebrate development. *Nat Rev Genet*. 2010;11(5):331–344.
4. Falk N, Lösl M, Schröder N, Gießl A. Specialized cilia in mammalian sensory systems. *Cells*. 2015;4(3):500–519.
5. Flock A, Duvall AJ III. The ultrastructure of the kinocilium of the sensory cells in the inner ear and lateral line organs. *J Cell Biol*. 1965;25:1–8.
6. Plotnikova OV, Pugacheva EN, Golemis EA. Primary cilia and the cell cycle. *Methods Cell Biol*. 2009;94:137–160.
7. Pedersen LB, Veland IR, Schrøder JM, Christensen ST. Assembly of primary cilia. *Dev Dyn*. 2008;237(8):1993–2006.
8. Sorokin SP. Reconstructions of centriole formation and ciliogenesis in mammalian lungs. *J Cell Sci*. 1968;3(2):207–230.
9. Iomini C, Tejada K, Mo W, Vaananen H, Piperno G. Primary cilia of human endothelial cells disassemble under laminar shear stress. *J Cell Biol*. 2004;164(6):811–817.
10. Ishikawa H, Kubo A, Tsukita S, Tsukita S. Odf2-deficient mother centrioles lack distal/subdistal appendages and the ability to generate primary cilia. *Nat Cell Biol*. 2005;7(5):517–524.
11. Beales PL, Bland E, Tobin JL, et al. IFT80, which encodes a conserved intraflagellar transport protein, is mutated in Jeune asphyxiating thoracic dystrophy. *Nat Genet*. 2007;39(6):727–729.
12. Brazelton WJ, Amundsen CD, Silflow CD, Lefebvre PA. The bld1 mutation identifies the *Chlamydomonas* osm-6 homolog as a gene required for flagellar assembly. *Curr Biol*. 2001;11(20):1591–1594.
13. Cole DG. The intraflagellar transport machinery of *Chlamydomonas reinhardtii*. *Traffic*. 2003;4(7):435–442.
14. Deane JA, Cole DG, Seeley ES, Diener DR, Rosenbaum JL. Localization of intraflagellar transport protein IFT52 identifies basal body transitional fibers as the docking site for IFT particles. *Curr Biol*. 2001;11(20):1586–1590.
15. Follit JA, Tuft RA, Fogarty KE, Pazour GJ. The intraflagellar transport protein IFT20 is associated with the Golgi complex and is required for cilia assembly. *Mol Biol Cell*. 2006;17(9):3781–3792.
16. Fujiwara M, Ishihara T, Katsura I. A novel WD40 protein, CHE-2, acts cell-autonomously in the formation of *C. elegans* sensory cilia. *Development*. 1999;126(21):4839–4848.
17. Haycraft CJ, Swoboda P, Taulman PD, Thomas JH, Yoder BK. The *C. elegans* homolog of the murine cystic kidney disease gene Tg737 functions in a ciliogenic pathway and is disrupted in osm-5 mutant worms. *Development*. 2001;128(9):1493–1505.
18. Houde C, Dickinson RJ, Houtzager VM, et al. Hippi is essential for node cilia assembly and Sonic hedgehog signaling. *Dev Biol*. 2006;300(2):523–533.
19. Huangfu D, Liu A, Rakeman AS, Murcia NS, Niswander L, Anderson KV. Hedgehog signalling in the mouse requires intraflagellar transport proteins. *Nature*. 2003;426(6962):83–87.
20. Pazour GJ, Dickert BL, Vucica Y, et al. Chlamydomonas IFT88 and its mouse homologue, polycystic kidney disease gene tg737, are required for assembly of cilia and flagella. *J Cell Biol*. 2000;151(3):709–718.
21. Perkins LA, Hedgecock EM, Thomson JN, Culotti JG. Mutant sensory cilia in the nematode *Caenorhabditis elegans*. *Dev Biol*. 1986;117(2):456–487.
22. Qin H, Rosenbaum JL, Barr MM. An autosomal recessive polycystic kidney disease gene homolog is involved in intraflagellar transport in *C. elegans* ciliated sensory neurons. *Curr Biol*. 2001;11(6):457–461.
23. Qin H, Wang Z, Diener D, Rosenbaum J. Intraflagellar transport protein 27 is a small G protein involved in cell-cycle control. *Curr Biol*. 2007;17(3):193–202.
24. Sun Z, Amsterdam A, Pazour GJ, Cole DG, Miller MS, Hopkins N. A genetic screen in zebrafish identifies cilia genes as a principal cause of cystic kidney. *Development*. 2004;131(16):4085–4093.
25. Tsujikawa M, Malicki J. Intraflagellar transport genes are essential for differentiation and survival of vertebrate sensory neurons. *Neuron*. 2004;42(5):703–716.
26. Graser S, Stierhof YD, Lavoie SB, et al. Cep164, a novel centriole appendage protein required for primary cilium formation. *J Cell Biol*. 2007;179(2):321–330.
27. Mikule K, Delaval B, Kaldis P, Jurcyzk A, Hergert P, Doxsey S. Loss of centrosome integrity induces p38-p53-p21-dependent G1-S arrest. *Nat Cell Biol*. 2007;9(2):160–170.
28. Matsuura K, Lefebvre PA, Kamiya R, Hirono M. Kinesin-II is not essential for mitosis and cell growth in *Chlamydomonas*. *Cell Motil Cytoskeleton*. 2002;52(4):195–201.
29. Marszalek JR, Ruiz-Lozano P, Roberts E, Chien KR, Goldstein LS. Situs inversus and embryonic ciliary morphogenesis defects in mouse mutants lacking the KIF3A subunit of kinesin-II. *Proc Natl Acad Sci U S A*. 1999;96(9):5043–5048.
30. Nonaka S, Tanaka Y, Okada Y, et al. Randomization of left-right asymmetry due to loss of nodal cilia generating leftward flow of extraembryonic fluid in mice lacking KIF3B motor protein. *Cell*. 1998;95(6):829–837.
31. Takeda S, Yonekawa Y, Tanaka Y, Okada Y, Nonaka S, Hirokawa N. Left-right asymmetry and kinesin superfamily protein KIF3A: new insights in determination of laterality and mesoderm induction by kif3A-/- mice analysis. *J Cell Biol*. 1999;145(4):825–836.
32. Rana AA, Barbera JP, Rodriguez TA, et al. Targeted deletion of the novel cytoplasmic dynein mD2LIC disrupts the embryonic organiser, formation of the body axes and specification of ventral cell fates. *Development*. 2004;131(20):4999–5007.
33. Tang Z, Lin MG, Stowe TR, et al. Autophagy promotes primary ciliogenesis by removing OFD1 from centriolar satellites. *Nature*. 2013;502(7470):254–257.
34. Singla V, Romaguera-Ros M, Garcia-Verdugo JM, Reiter JF. Ofd1, a human disease gene, regulates the length and distal structure of centrioles. *Dev Cell*. 2010;18(3):410–424.
35. Kasahara K, Kawakami Y, Kiyono T, et al. Ubiquitin-proteasome system controls ciliogenesis at the initial step of axoneme extension. *Nat Commun*. 2014;5:5081.
36. Gerhardt C, Lier JM, Burmuhl S, et al. The transition zone protein Rpgrip1l regulates proteasomal activity at the primary cilium. *J Cell Biol*. 2015;210(1):115–133.
37. Lutz MS, Burk RD. Primary cilium formation requires von Hippel-Lindau gene function in renal-derived cells. *Cancer Res*. 2006;66(14):6903–6907.
38. Schermer B, Ghenoiu C, Bartram M, et al. The von Hippel-Lindau tumor suppressor protein controls ciliogenesis by orienting microtubule growth. *J Cell Biol*. 2006;175(4):547–554.
39. Thoma CR, Frew IJ, Krek W. The VHL tumor suppressor: riding tandem with GSK3beta in primary cilium maintenance. *Cell Cycle*. 2007;6(15):1809–1813.
40. Xu J, Li H, Wang B, et al. VHL inactivation induces HEF1 and Aurora kinase A. *J Am Soc Nephrol*. 2010;21(12):2041–2046.
41. Pampliega O, Orhon I, Patel B, et al. Functional interaction between autophagy and ciliogenesis. *Nature*. 2013;502(7470):194–200.
42. Pan J, Wang Q, Snell WJ. An aurora kinase is essential for flagellar disassembly in *Chlamydomonas*. *Dev Cell*. 2004;6(3):445–451.
43. Mergen M, Engel C, Muller B, et al. The nephronophthisis gene product NPHP2/Inversin interacts with Aurora A and interferes with HDAC6-mediated cilia disassembly. *Nephrol Dial Transplant*. 2013;28(11):2744–2753.
44. Wang G, Chen Q, Zhang X, et al. PCM1 recruits Plk1 to the pericentriolar matrix to promote primary cilia disassembly before mitotic entry. *J Cell Sci*. 2013;126(pt 6):1355–1365.

45. Miyamoto T, Hosoba K, Ochiai H, et al. The microtubule-depolymerizing activity of a mitotic kinesin protein KIF2A drives primary cilia disassembly coupled with cell proliferation. *Cell Rep*. 2015;10:664–673.
46. Lee KH, Johmura Y, Yu LR, et al. Identification of a novel Wnt5a-CK1varepsilon-Dvl2-Plk1-mediated primary cilia disassembly pathway. *EMBO J*. 2012;31(14):3104–3117.
47. Seeger-Nukpezah T, Liebau MC, Hopker K, et al. The centrosomal kinase Plk1 localizes to the transition zone of primary cilia and induces phosphorylation of nephrocystin-1. *PLoS One*. 2012;7(6):e38838.
48. Pugacheva EN, Jablonski SA, Hartman TR, Henske EP, Golemis EA. HEF1-dependent Aurora A activation induces disassembly of the primary cilium. *Cell*. 2007;129(7):1351–1363.
49. Tsang WY, Bossard C, Khanna H, et al. CP110 suppresses primary cilia formation through its interaction with CEP290, a protein deficient in human ciliary disease. *Dev Cell*. 2008;15(2):187–197.
50. Huang K, Diener DR, Rosenbaum JL. The ubiquitin conjugation system is involved in the disassembly of cilia and flagella. *J Cell Biol*. 2009;186(4):601–613.
51. Hildebrandt F, Benzing T, Katsanis N. Ciliopathies. *N Engl J Med*. 2011;364(16):1533–1543.
52. Waters AM, Beales PL. Ciliopathies: an expanding disease spectrum. *Pediatr Nephrol*. 2011;26(7):1039–1056.
53. Badano JL, Mitsuma N, Beales PL, Katsanis N. The ciliopathies: an emerging class of human genetic disorders. *Annu Rev Genomics Hum Genet*. 2006;7:125–148.
54. Hildebrandt F, Attanasio M, Otto E. Nephronophthisis: disease mechanisms of a ciliopathy. *J Am Soc Nephrol*. 2009;20(1):23–35.
55. Ibanez-Tallon I, Heintz N, Omran H. To beat or not to beat: roles of cilia in development and disease. *Hum Mol Genet*. 2003;12(Spec No 1):R27–R35.
56. Boon M, Jorissen M, Proesmans M, De Boeck K. Primary ciliary dyskinesia, an orphan disease. *Eur J Pediatr*. 2013;172(2):151–162.
57. Mukhopadhyay S, Jackson PK. Cilia, tubby mice, and obesity. *Cilia*. 2013;2:1.
58. Briscoe J, Therond PP. The mechanisms of Hedgehog signalling and its roles in development and disease. *Nat Rev Mol Cell Biol*. 2013;14(7):416–429.
59. Rohatgi R, Milenkovic L, Scott MP. Patched1 regulates hedgehog signaling at the primary cilium. *Science*. 2007;317(5836):372–376.
60. Wong SY, Reiter JF. The primary cilium at the crossroads of mammalian hedgehog signaling. *Curr Top Dev Biol*. 2008;85:225–260.
61. Goetz SC, Ocbina PJ, Anderson KV. The primary cilium as a Hedgehog signal transduction machine. *Methods Cell Biol*. 2009;94:199–222.
62. Huang P, Schier AF. Dampened Hedgehog signaling but normal Wnt signaling in zebrafish without cilia. *Development*. 2009;136(18):3089–3098.
63. Oishi I, Kawakami Y, Raya A, Callol-Massot C, Izpisúa Belmonte JC. Regulation of primary cilia formation and left-right patterning in zebrafish by a noncanonical Wnt signaling mediator, duboraya. *Nat Genet*. 2006;38(11):1316–1322.
64. Gerdes JM, Liu Y, Zaghloul NA, et al. Disruption of the basal body compromises proteasomal function and perturbs intracellular Wnt response. *Nat Genet*. 2007;39(11):1350–1360.
65. Corbit KC, Shyer AE, Dowdle WE, et al. Kif3a constrains beta-catenin-dependent Wnt signalling through dual ciliary and non-ciliary mechanisms. *Nat Cell Biol*. 2008;10(1):70–76.
66. Logan CY, Nusse R. The Wnt signaling pathway in development and disease. *Annu Rev Cell Dev Biol*. 2004;20:781–810.
67. Komiya Y, Habas R. Wnt signal transduction pathways. *Organogenesis*. 2008;4(2):68–75.
68. Wagner U, Brownlees J, Irving NG, Lucas FR, Salinas PC, Miller CC. Overexpression of the mouse dishevelled-1 protein inhibits GSK-3beta-mediated phosphorylation of tau in transfected mammalian cells. *FEBS Lett*. 1997;411(2–3):369–372.
69. Cliffe A, Hamada F, Bienz M. A role of Dishevelled in relocating Axin to the plasma membrane during wingless signaling. *Curr Biol*. 2003;13(11):960–966.
70. Schlessinger K, Hall A, Tolwinski N. Wnt signaling pathways meet Rho GTPases. *Genes Dev*. 2009;23(3):265–277.
71. Balakumar P, Jagadeesh G. Potential cross-talk between (pro)renin receptors and Wnt/frizzled receptors in cardiovascular and renal disorders. *Hypertens Res*. 2011;34(11):1161–1170.
72. Ocbina PJ, Tuson M, Anderson KV. Primary cilia are not required for normal canonical Wnt signaling in the mouse embryo. *PLoS One*. 2009;4(8):e6839.
73. Simons M, Gloy J, Ganner A, et al. Inversin, the gene product mutated in nephronophthisis type II, functions as a molecular switch between Wnt signaling pathways. *Nat Genet*. 2005;37(5):537–543.
74. Bergmann C, Fliegauf M, Bruchle NO, et al. Loss of nephrocystin-3 function can cause embryonic lethality, Meckel-Gruber-like syndrome, situs inversus, and renal-hepatic-pancreatic dysplasia. *Am J Hum Genet*. 2008;82(4):959–970.
75. Jonassen JA, San Agustin J, Follit JA, Pazour GJ. Deletion of IFT20 in the mouse kidney causes misorientation of the mitotic spindle and cystic kidney disease. *J Cell Biol*. 2008;183(3):377–384.
76. Wiens CJ, Tong Y, Esmail MA, et al. Bardet-Biedl syndrome-associated small GTPase ARL6 (BBS3) functions at or near the ciliary gate and modulates Wnt signaling. *J Biol Chem*. 2010;285(21):16218–16230.
77. Lancaster MA, Schroth J, Gleeson JG. Subcellular spatial regulation of canonical Wnt signalling at the primary cilium. Nat Cell Biol. 2011;13(6):700–707.
78. Lancaster MA, Louie CM, Silhavy JL, et al. Impaired Wnt-beta-catenin signaling disrupts adult renal homeostasis and leads to cystic kidney ciliopathy. *Nat Med*. 2009;15(9):1046–1054.
79. Schneider L, Clement CA, Teilmann SC, et al. PDGFRalphaalpha signaling is regulated through the primary cilium in fibroblasts. *Curr Biol*. 2005;15(20):1861–1866.
80. Clement DL, Mally S, Stock C, et al. PDGFRalpha signaling in the primary cilium regulates NHE1-dependent fibroblast migration via coordinated differential activity of MEK1/2-ERK1/2-p90RSK and AKT signaling pathways. *J Cell Sci*. 2013;126(pt 4):953–965.
81. Liang J, Zubovitz J, Petrocelli T, et al. PKB/Akt phosphorylates p27, impairs nuclear import of p27 and opposes p27-mediated G1 arrest. *Nat Med*. 2002;8(10):1153–1160.
82. Katayama K, Nakamura A, Sugimoto Y, Tsuruo T, Fujita N. FOXO transcription factor-dependent p15(INK4b) and p19(INK4d) expression. *Oncogene*. 2008;27(12):1677–1686.
83. Schneider L, Cammer M, Lehman J, et al. Directional cell migration and chemotaxis in wound healing response to PDGF-AA are coordinated by the primary cilium in fibroblasts. *Cell Physiol Biochem*. 2010;25(2–3):279–292.
84. Danilov AI, Gomes-Leal W, Ahlenius H, Kokaia Z, Carlemalm E, Lindvall O. Ultrastructural and antigenic properties of neural stem cells and their progeny in adult rat subventricular zone. *Glia*. 2009;57(2):136–152.
85. Klinghoffer RA, Hamilton TG, Hoch R, Soriano P. An allelic series at the PDGFalphaR locus indicates unequal contributions of distinct signaling pathways during development. *Dev Cell*. 2002;2(1):103–113.
86. Soriano P. The PDGF alpha receptor is required for neural crest cell development and for normal patterning of the somites. *Development*. 1997;124(14):2691–2700.
87. Clement CA, Ajbro KD, Koefoed K, et al. TGF-beta signaling is associated with endocytosis at the pocket region of the primary cilium. *Cell Rep*. 2013;3(6):1806–1814.
88. Miraoui H, Marie PJ. Fibroblast growth factor receptor signaling crosstalk in skeletogenesis. *Sci Signal*. 2010;3(146):re9.
89. Ma R, Li WP, Rundle D, Kong J, Akbarali HI, Tsiokas L. PKD2 functions as an epidermal growth factor-activated plasma membrane channel. *Mol Cell Biol*. 2005;25(18):8285–8298.
90. Berbari NF, Johnson AD, Lewis JS, Askwith CC, Mykytyn K. Identification of ciliary localization sequences within the third intracellular loop of G protein-coupled receptors. *Mol Biol Cell*. 2008;19(4):1540–1547.
91. Iwanaga T, Miki T, Takahashi-Iwanaga H. Restricted expression of somatostatin receptor 3 to primary cilia in the pancreatic islets and adenohypophysis of mice. *Biomed Res*. 2011;32(1):73–81.
92. Seo S, Guo DF, Bugge K, Morgan DA, Rahmouni K, Sheffield VC. Requirement of Bardet-Biedl syndrome proteins for leptin receptor signaling. *Hum Mol Genet*. 2009;18(7):1323–1331.
93. Han YM, Kang GM, Byun K, et al. Leptin-promoted cilia assembly is critical for normal energy balance. *J Clin Invest*. 2014;124(5):2193–2197.
94. Zhu D, Shi S, Wang H, Liao K. Growth arrest induces primary-cilium formation and sensitizes IGF-1-receptor signaling during differentiation induction of 3T3-L1 preadipocytes. *J Cell Sci*. 2009;122(pt 15):2760–2768.
95. Wu J, Du H, Wang X, Mei C, Sieck GC, Qian Q. Characterization of primary cilia in human airway smooth muscle cells. *Chest*. 2009;136(2):561–570.
96. Christensen ST, Clement CA, Satir P, Pedersen LB. Primary cilia and coordination of receptor tyrosine kinase (RTK) signalling. *J Pathol*. 2012;226(2):172–184.
97. Shiba D, Yamaoka Y, Hagiwara H, Takamatsu T, Hamada H, Yokoyama T. Localization of Inv in a distinctive intraciliary compartment requires the C-terminal ninein-homolog-containing region. *J Cell Sci*. 2009;122(pt 1):44–54.
98. Morgan D, Eley L, Sayer J, et al. Expression analyses and interaction with the anaphase promoting complex protein Apc2 suggest a role for inversin in primary cilia and involvement in the cell cycle. *Hum Mol Genet*. 2002;11(26):3345–3350.
99. Kikuchi K, Niikura Y, Kitagawa K, Kikuchi A. Dishevelled, a Wnt signalling component, is involved in mitotic progression in cooperation with Plk1. *EMBO J*. 2010;29(20):3470–3483.
100. Lienkamp S, Ganner A, Walz G. Inversin, Wnt signaling and primary cilia. *Differentiation*. 2012;83(2):S49–S55.
101. Michaud EJ, Yoder BK. The primary cilium in cell signaling and cancer. *Cancer Res*. 2006;66(13):6463–6467.
102. Han YG, Kim HJ, Dlugosz AA, Ellison DW, Gilbertson RJ, Alvarez-Buylla A. Dual and opposing roles of primary cilia in medulloblastoma development. *Nat Med*. 2009;15(9):1062–1065.
103. Moser JJ, Fritzler MJ, Rattner JB. Primary ciliogenesis defects are associated with human astrocytoma/glioblastoma cells. *BMC Cancer*. 2009;9:448.

104. Seeley ES, Carrière C, Goetze T, Longnecker DS, Korc M. Pancreatic cancer and precursor pancreatic intraepithelial neoplasia lesions are devoid of primary cilia. *Cancer Res*. 2009;69(2):422–430.
105. Schraml P, Frew IJ, Thoma CR, et al. Sporadic clear cell renal cell carcinoma but not the papillary type is characterized by severely reduced frequency of primary cilia. *Mod Pathol*. 2009;22(1):31–36.
106. Wong SY, Seol AD, So PL, et al. Primary cilia can both mediate and suppress Hedgehog pathway-dependent tumorigenesis. *Nat Med*. 2009;15(9):1055–1061.
107. Yuan K, Frolova N, Xie Y, et al. Primary cilia are decreased in breast cancer: analysis of a collection of human breast cancer cell lines and tissues. *J Histochem Cytochem*. 2010;58(10):857–870.
108. Hassounah NB, Bunch TA, McDermott KM. Molecular pathways: the role of primary cilia in cancer progression and therapeutics with a focus on Hedgehog signaling. *Clin Cancer Res*. 2012;18(9):2429–2435.
109. Hassounah NB, Nagle R, Saboda K, Roe DJ, Dalkin BL, McDermott KM. Primary cilia are lost in preinvasive and invasive prostate cancer. *PLoS One*. 2013; 8(7):e68521.
110. Menzl I, Lebeau L, Pandey R, et al. Loss of primary cilia occurs early in breast cancer development. *Cilia*. 2014;3:7.
111. Basten SG, Willekers S, Vermaat JS, et al. Reduced cilia frequencies in human renal cell carcinomas versus neighboring parenchymal tissue. *Cilia*. 2013;2(1):2.
112. Snedecor ER, Sung CC, Moncayo A, et al. Loss of primary cilia in melanoma cells is likely independent of proliferation and cell cycle progression. *J Invest Dermatol*. 2015;135(5):1456–1458.
113. Kim J, Dabiri S, Seeley ES. Primary cilium depletion typifies cutaneous melanoma in situ and malignant melanoma. *PLoS One*. 2011;6(11):e27410.
114. Saran A. Basal cell carcinoma and the carcinogenic role of aberrant Hedgehog signaling. *Future Oncol*. 2010;6(6):1003–1014.
115. DeRouen MC, Zhen H, Tan SH, Williams S, Marinkovich MP, Oro AE. Laminin-511 and integrin beta-1 in hair follicle development and basal cell carcinoma formation. *BMC Dev Biol*. 2010;10:112.
116. Otsuka A, Dreier J, Cheng PF, et al. Hedgehog pathway inhibitors promote adaptive immune responses in basal cell carcinoma. *Clin Cancer Res*. 2015;21(6): 1289–1297.
117. Barakat MT, Humke EW, Scott MP. Kif3a is necessary for initiation and maintenance of medulloblastoma. *Carcinogenesis*. 2013;34(6):1382–1392.
118. Gate D, Danielpour M, Bannykh S, Town T. Characterization of cancer stem cells and primary cilia in medulloblastoma. *CNS Neurol Disord Drug Targets*. 2015; 14(5):600–611.
119. Bailey JM, Mohr AM, Hollingsworth MA. Sonic hedgehog paracrine signaling regulates metastasis and lymphangiogenesis in pancreatic cancer. *Oncogene*. 2009;28(40):3513–3525.
120. Shih AH, Holland EC. Platelet-derived growth factor (PDGF) and glial tumorigenesis. *Cancer Lett*. 2006;232(2):139–147.
121. Seeley ES, Nachury MV. The perennial organelle: assembly and disassembly of the primary cilium. *J Cell Sci*. 2010;123(pt 4):511–518.
122. Egeberg DL, Lethan M, Manguso R, et al. Primary cilia and aberrant cell signaling in epithelial ovarian cancer. *Cilia*. 2012;1(1):15.
123. Thoma CR, Frew IJ, Hoerner CR, Montani M, Moch H, Krek W. pVHL and GSK3beta are components of a primary cilium-maintenance signalling network. *Nat Cell Biol*. 2007;9(5):588–595.
124. Boehlke C, Kotsis F, Patel V, et al. Primary cilia regulate mTORC1 activity and cell size through Lkb1. *Nat Cell Biol*. 2010;12(11):1115–1122.
125. Courtois-Cox S, Jones SL, Cichowski K. Many roads lead to oncogene-induced senescence. *Oncogene*. 2008;27(20):2801–2809.
126. Breslin L, Prosser SL, Cuffe S, Morrison CG. Ciliary abnormalities in senescent human fibroblasts impair proliferative capacity. *Cell Cycle*. 2014;13(17): 2773–2779.
127. Gallage S, Gil J. Primary cilia and senescence: a sensitive issue. *Cell Cycle*. 2014;13(17):2653–2654.
128. Breslin L. The Primary Cilium as a Regulator of Cellular Senescence in Human Fibroblasts. [NUI Galway Thesis]; 2014:1–165.
129. Ramachandran I, Ganapathy V, Gillies E, et al. Wnt inhibitory factor 1 suppresses cancer stemness and induces cellular senescence. *Cell Death Dis*. 2014; 5:e1246.
130. Liu S, Liu S, Wang X, et al. The PI3K-Akt pathway inhibits senescence and promotes self-renewal of human skin-derived precursors in vitro. *Aging Cell*. 2011;10(4):661–674.
131. Praetorius HA, Spring KR. A physiological view of the primary cilium. *Annu Rev Physiol*. 2005;67:515–529.
132. Liem KF Jr, Ashe A, He M, et al. The IFT-A complex regulates Shh signaling through cilia structure and membrane protein trafficking. *J Cell Biol*. 2012;197(6):789–800.
133. Chang CF, Schock EN, O'Hare EA, et al. The cellular and molecular etiology of the craniofacial defects in the avian ciliopathic mutant talpid2. *Development*. 2014;141(15):3003–3012.
134. Hardy T, Lee M, Hames RS, et al. Multisite phosphorylation of C-Nap1 releases it from Cep135 to trigger centrosome disjunction. *J Cell Sci*. 2014;127(pt 11): 2493–2506.
135. Wang G, Jiang Q, Zhang C. The role of mitotic kinases in coupling the centrosome cycle with the assembly of the mitotic spindle. *J Cell Sci*. 2014;127(pt 19): 4111–4122.
136. Huang C, Jacobson K, Schaller MD. MAP kinases and cell migration. *J Cell Sci*. 2004;117(pt 20):4619–4628.
137. Sun Y, Liu WZ, Liu T, Feng X, Yang N, Zhou HF. Signaling pathway of MAPK/ERK in cell proliferation, differentiation, migration, senescence and apoptosis. *J Recept Signal Transduct Res*. 2015;35(6):600–604.