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# The role of the cilium in hereditary tumor predisposition syndromes

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**Abstract**. The primary cilium is a highly conserved cell organelle that is closely connected to processes involved in cell patterning and replication. Amongst their many functions, cilia act as "signal towers" through which cell-cell signaling cascades pass. Dysfunction of cilia or the myriad processes that are connected with cilium function can lead to disease. Due to the sheer number of cellular processes that at some point involve the primary cilium, the effects of misregulation are highly heterogeneous between different cell populations. However, because of the importance of primary cilia in the development, growth, patterning and orientation of cells and tissues, a common thread has emerged in which defective cilia can lead to disorganization, which can contribute to the growth of neoplasms, including cancer and pre-cancerous phenotypes. Because cilia are so vital for signaling during cell replication and the cell fate decisions that are important in childhood growth, symptoms often arise early in life. Here we review recent work connecting misregulation of the primary cilium with tumor formation in a variety of tissues in the developing body, with a particular focus on the syndromes in which classic tumor genes are mutated, including von Hippel-Lindau disease (OMIM 193300), adenomatous polyposis coli (OMIM 175100), tuberous sclerosis (OMIM 191100) and Birt-Hogg-Dubé syndrome (OMIM 135150). Timely diagnosis of these syndromes is essential for entry into appropriate screening protocols, which have been shown to effectively prolong life expectancy in these cohorts of patients.

Keywords: Cilia, von Hippel-Lindau disease, cyst, renal cell carcinoma, medulloblastoma

## 1. Introduction

The primary cilium, an antenna-like structure found on almost all mammalian cells, was once considered to be a mostly unimportant vestigial organelle but has now emerged as a vital player in a number of cellular processes. Cilia respond to physical and chemical signals to regulate critical signal transduction pathways; for example, cilia are both negative and positive regulators of the hedgehog pathway [1]. Many of these signaling pathways are crucial in proliferation and patterning of developing tissue. Because of their importance in growth and development at all levels, from single cells to tissue organization to the positioning of entire organs, misregulation of primary cilia is now widely acknowledged to be an important mechanism in the biology of developmental and degenerative disorders. Emerging data likewise indicates that cilia participate in tumorigenesis as well. Here we will focus on tumor types affecting children and the pathophysiology of inherited tumor syndromes implicated with cilia dysfunction.

The primary cilium is central to pleiotropic effects in growth and patterning of cells, tissues and organisms both through its involvement in a wide variety of cellular signaling and sensory pathways and by its direct involvement in cell division. In order to have

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proper ciliary function, a cell must be able to assemble, maintain, and then disassemble its primary cilium. Cells with malfunctioning cilia display varied phenotypes including the disruption of many signaling pathways, aberrations in the cell cycle and intracellular trafficking defects. A mutation which affects the structure or function of cilia can therefore lead to downstream changes in the phenotype of the cell population and even the tissue, including anomalies in cell polarization, cell fate determination, cell metabolism and cell division as well as an inability to properly respond to extracellular signals and changes in the internal environment such as DNA damage [2–4]. Because of this wide array of functions, many outcomes of ciliary dysfunction are possible including the development of pathogenic phenotypes in humans as well as model organisms [5–7]. Diseases arising from misregulation of cilia formation, maintenance or disassembly, known collectively as ciliopathies, can be pathologically mild or so severe that they cause neonatal death. The ciliopathies include a number of inherited developmental disorders that are directly caused by, or involve, misregulation of cilia, as well as different tumor types, cancerous diseases and syndromes. In this context, some pediatric tumors, such as Wilms' tumor of the kidney, can be viewed as a developmental disorder as it arises from mutations in survival and developmental pathways that cause these processes to be co-opted to provide survival strategies for tumor cells [8, 9]. Currently, the role of primary cilia in tumorigenesis and cancer-related processes is not well understood, but recent data indicate that cell cycle misregulation caused by defective cilia as well as associated signaling irregularities contributes to tumor formation [10, 11].

## 2. The primary cilium and cell cycle regulation

The essential biology of the primary cilium (which will not be covered by this review as it has already been well-reviewed elsewhere [1, 12–14]) links it inextricably to the cell cycle through the basal body. The cilium is formed during the stationary G0-phase of the cell cycle. Prior to cell division, the ciliary axoneme must be disassembled. The basal body then decouples from the plasma membrane to function as the centrosome anchoring the mitotic spindle. The centriole also forms the microtubule organizing center which nucleates the microtubule skeleton. Thus, proliferation and ciliation are mutually exclusive; a cell cannot divide and remain ciliated. This makes the disassembly of cilia a crucial checkpoint in the cell cycle (Fig. 1). One might expect that in the absence of cilia, this checkpoint is abolished and a cell would divide at a higher rate. Indeed, loss of cilia increases tumor incidence in murine models of basal cell carcinoma and medulloblastoma, both of which will be discussed in greater detail below [15–18]. A number of efforts have been made to catalogue cilia presence in human cancers: pancreatic cancer, renal cell carcinoma (RCC), cholangiocarcinoma, melanoma, ovarian cancer, prostate cancer, and breast cancer. All of these neoplasms feature significantly fewer cilia [19–27]. In addition, a number of proteins that are closely related to ciliogenesis and ciliary function are also known to have direct roles in cell cycle control. For example, reduction of cellular levels of Ift88, an intraflagellar transport protein required for the formation of the primary cilium, induces cell cycle progression *in vitro*. *IFT88* mutations have been found in human postnatal lethal Meckel-Gruber syndrome (OMIM 249000) patients. Hypomorphic mutations in have also been shown to cause a ciliopathy in mice [28]; *orpk/Ift88* mutant mice also display epithelial cell hyperproliferation [21, 29, 30]. Interestingly, recent data

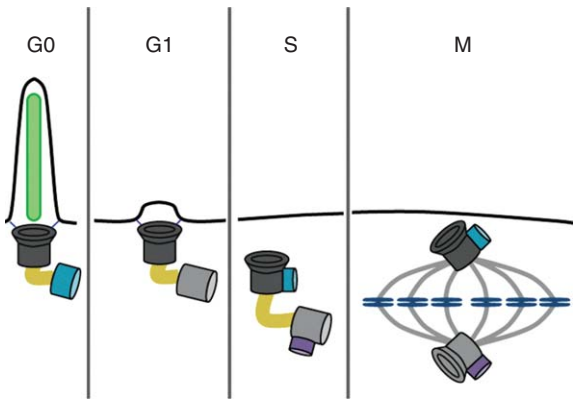


Fig. 1. The presence of the primary cilium and cell replication are mutually exclusive. In G0-phase of the cell cycle, a fully formed cilium is present. As the cell progresses into G1-phase, the ciliary axoneme must be disassembled. The centrosome can then decouple from the plasma membrane allowing it to migrate into the cell where it will be replicated during S-phase. This process is required for normal mitotic spindle formation during mitosis. Mutations, which inhibit the construction of the ciliary axoneme or the coupling of the centrosome to the cell membrane may hypothetically promote replication by removing the need for deconstruction of the cilium before replication. Mutations affecting the centrosome itself may also interfere with proper mitotic spindle organization.

suggest that modulation of retinoblastoma signaling, (responsible for entry into the S-phase of the cell cycle), by the von Hippel-Lindau tumor suppressor protein (pVHL) is related to overall survival in both sporadic and hereditary RCC [31]. Collectively, these data support the hypothesis that loss of cilia may promote cancer development in certain tissues. In particular, the localization of classic tumor suppressor proteins, typically mutated in familial tumor predisposition syndromes, to the cilium raises interesting questions concerning the relation between cilia and tumor development. For example, canonical tumor suppressor proteins such as pVHL, liver kinase B (LKB) 1, tuberous sclerosis (TSC) 1, TSC2 and adenomatous polyposis coli (APC) have been shown to localize to ciliary axonemes and/or basal bodies in ciliated cells [32]. Many of these proteins bind microtubules and thus have overlapping roles during interphase and mitosis. However, although many ciliopathies involve cell cycle defects, not all ciliopathies involve hyperplasia. Therefore, while the disassembly of cilia is required for cell proliferation, it will not always suffice to cause an increased rate of cell division on its own. While tumor formation frequently results from multiple germline and somatic mutations acquired over the course of many years, many ciliopathies are diagnosed in childhood. This may attribute to the absence of tumor prevalence in ciliopathy patients when diagnosed. Thus, while not all genetic mutations that cause a loss of ciliation will lead to cancer, many of them have the potential to cause, or at least contribute to, a proliferative phenotype, including cyst formation.

## 3. Ciliopathy-related renal cysts

Data collected on ciliopathies support the hypothesis that misregulation of cilia can be a cause of neoplasm formation. A common feature of kidney diseases associated with the loss of function of cilia-associated proteins is the development of multiple fluid-filled cysts along the renal nephron. These cysts develop in part due to misaligned centrosomes that cause misoriented cell division (Fig. 2). Many ciliopathies share this feature, including autosomal dominant (AD) (OMIM # 173900) or recessive polycystic kidney disease (OMIM # 263200), Bardet-Biedl syndrome (OMIM # 209900) and Joubert syndrome (OMIM #213300). These cysts progressively impair renal function leading to the development of end-stage renal disease [14, 33]. They stain positive for markers characteristic of altered proliferation, wingless-related integration site (Wnt) signaling or proliferation (e.g. nuclear β-catenin [34]) but only rarely progress into renal tumors. Intriguingly, disorganization and improper regulation of the centrosome is also considered a hallmark of multiple cancer types [35], many of which also display neoplasm formation of this kind. Because the centrosome also functions as the basal body, many proteins that localize to the basal body and other parts of the cilium while the cell is ciliated in the G0-phase of the cell cycle contribute to the function and regulation of the centrosome during mitosis. For example, before cell division can occur, the mother centriole must duplicate itself to form the daughter centriole. This pair of centrioles is then used to form the spindle poles during mitosis (see previous section). The process of centriole duplication is controlled by the protein polo-like kinase 4 (PLK4) which localizes to the centrosome. Abnormal expression of PLK4 in some cancer types has been implicated in genomic instability that might contribute to tumor formation. Overexpression of PLK4 can cause extra centrioles to be produced, leading to malformed mitotic spindles and aneuploidy of daughter cells after division [36, 37]. The incidence of renal cysts in many syndromic ciliopathies and the incrimination of certain proteins which normally localize to the cilium and regulate or participate in cell division as assisting tumor growth when malfunctioning has led to the hypothesis

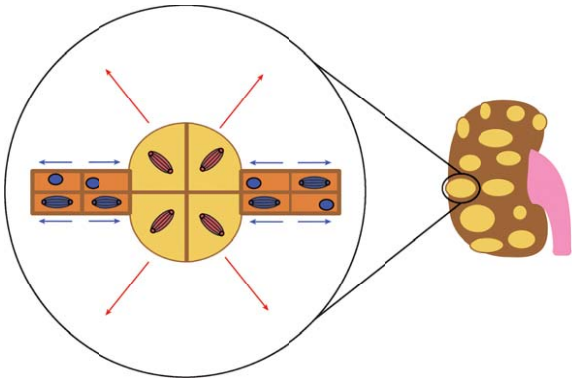

Fig. 2. Cells dividing out of phase with the rest of the tissue can produce cysts. In this example, most of the renal cells are dividing in a similar direction (horizontal arrows). A mutation causes division to proceed out of the plane of the tissue, leading to a bulge. This bulge eventually becomes a renal cyst and widespread expression of this phenotype leads to a polycystic kidney. Cysts in other tissues can develop in a similar way.

that renal cysts found in ciliopathy patients are a premalignant stage and that renal cancers might develop from such cysts [38]. In some cases, such as in the case of the *VHL* gene, which is itself an oncogene due to its vital role in regulating hypoxia signaling, a mutation that causes cysts might also directly contribute to the development of cancer. In other cases, a second (or more) somatic mutations may be required in order to fully transform cells.

## 4. Cilia misregulation and tumorigenesis

The natural course of any inherited tumor syndrome varies from individual to individual, with symptoms ranging from very mild to quite severe, occurring only when an independent somatic mutation inactivates the wild-type allele producing a cell with biallelic inactivation of the tumor suppressor in question [39]. At this point, the direct contribution of mutations which cause cilia dysfunction to tumorigenesis is incompletely understood and remains highly contentious. The connection between the primary cilium and the cell cycle, on the other hand, is both fundamental and undebatable. As mentioned above, cystic growths are a common symptom of ciliopathies and cyst development often occurs in hereditary cancer syndromes. In addition, some tumor types, a selection of which will be covered below, have also been shown to have reduced ciliary frequency, cilium function or signaling. However, cilia loss does not always correlate with tumorigenesis. Importantly, several studies on other tumor types, such as subsets of basal cell carcinoma and medulloblastoma, have indicated that in these tumors cilia are actually retained. As we discuss in greater detail below, the oncogenic mutations in these tumors are driven by members of the hedgehog signaling network, which is dependent on normal ciliary function to successfully generate downstream transcriptional activators and repressors [15, 16]. However, as we have previously mentioned, in other subsets of these same tumor types with different genetic backgrounds, cilia act as tumor suppressors. The ability of the cilium to act as both a tumor suppressor and an oncogenic force in the same tissues and even among the same tumor types shows the context-specificity of the cellular response to the loss of proper ciliary functions. Given the heterogeneity of the proteins involved in ciliation, the true extent of the role of misregulation of cilia in tumorigenesis remains to be completely elucidated. Below we review some of the cancer syndromes and tumor types in which there is any data with regard to the role of the primary cilium and discuss the emerging importance of ciliary malfunction in hereditary cancer syndromes and precursor cell populations (Table 1).

### *4.1. VHL*

VHL disease is a rare (1:36,000 incidence) genetic condition that predisposes patients to tumor formation

Table 1
Clinical features of hereditary tumor predisposition syndromes associated with ciliary malfunction

| Disease/syndrome | Frequency | Type of inheritance | Earliest manifestations | Clinical features |
|---|---|---|---|---|
| von Hippel-Lindau disease | 1:36,000 | Autosomal dominant | Retinal hemangioblastomas | Hemangioblastomas of the brain, spinal cord and retina, renal cell carcinomas, pheochromocytomas, neuroendocrine tumors, endolymphatic sac tumors, pancreatic, epididymal, broad ligament and renal cysts |
| Adenomatous polyposis coli- related disease | 1:7,000–1:22,000 | Autosomal dominant | Colonic polyps | Colonic polyps, polyps in stomach and intestines, osteomas and dental anomalies, congenital hypertrophy of the retinal pigment epithelium, soft tissue tumors, desmoid tumors |
| Tuberous sclerosis | 1:6,000 | Autosomal dominant | Highly variable, skin lesions are the most common symptom | Hypomelanotic macules, facial angiofibromas, shagreen patches, fibrous facial plaques, ungual fibromas, angiomyolipomas, kidney cysts, renal cell carcinomas, rhabdomyomas, heart arrhythmias, lymphangioleiomyomatosis |
| Birt-Hogg-Dubé syndrome | 1:200,000 | Autosomal dominant | Fibrofolliculomas | Fibrofolliculomas, trichodiscomas/angiofibromas, perifollicular fibromas and acrochordons, pulmonary cysts, pneumothorax, renal tumors |

in a variety of tissues. Typically, the first tumors observed in children or young adults are retinal hemangioblastomas, although cerebellar hemangioblastomas and pheochrocytomas have also been frequently reported in childhood and adolescence. RCCs occur in 70% of patients and represent the most common cause of mortality. Primary care physicians should refer any pediatric patient with a typical VHL tumor for genetic testing. RCCs occurring in individuals under the age of 45 yr are increasingly being referred for genetic testing as well because about 10% of them will carry germline *VHL* mutations and require additional screening for other tumors. Clinical diagnosis can be made following standardized guidelines and involves the presence of two tumors in an affected individual or one tumor and a family history of VHL. VHL disease is caused by mutations in the *VHL* gene that encodes the pVHL tumor suppressor protein. VHL disease is inherited in an AD manner and the majority of patients (80%) have an affected parent. Molecular diagnosis via sequencing of the *VHL* gene is the preferred method as this will detect the disease causing mutation in >90% of patients [40].

Under physiological circumstances, pVHL is an E3 ubiquitin ligase, responsible for the regulation of HIF-alpha transcription factors that modulate the cell's response to low oxygen levels in the body (hypoxia). In the absence of functional pVHL, pseudohypoxia signaling stabilizes HIF-alpha and causes aberrant induction of a wide variety of target genes, including pro-survival and vasoproliferative genes that contribute to tumor formation. In addition, the most common form of renal cancer, clear cell RCC (ccRCC), is always associated with mutations or loss of the *VHL* gene in humans [41]. For an in depth review of pVHL and HIF signaling in renal cancer, please see [42]. Both pVHL and HIF1-alpha have been shown to be required for the maintenance of ciliation *in vitro* [43–48]. In addition, RCC tumors lacking functional pVHL display reduced ciliary frequency compared with neighboring tissues [20, 22]. pVHL localizes to cilia and interacts with the ciliary motor kinesin-2 [49, 50]. Although the loss of pVHL function is not sufficient to inhibit ciliogenesis, it does interfere with normal cilia dynamics and (mechano)-sensory roles [48, 51–53]. *In vivo* studies using inducible Cre-Lox deletion of *Vhl, Gsk3β, Pten* and *Tp53* in renal tubules in mice furthermore indicate that the combined loss of *Vhl* and *Gsk3β* or *Pten* or *Tp53* (second site modifiers), but not any of these genetic lesions alone, results in a reduction of renal tubule ciliogenesis and facilitates kidney cyst development in the same manner as described above for ciliopathies [54–57] (Fig. 3). However, mice with loss of *Vhl* and/or *Gsk3β* in their kidney epithelium do not develop renal tumors, indicating that, at least in mice, additional somatic mutations are necessary for tumor formation. In humans however, deep sequencing of tumors has shown that *VHL* mutations or deletions are ubiquitous events in all sur-

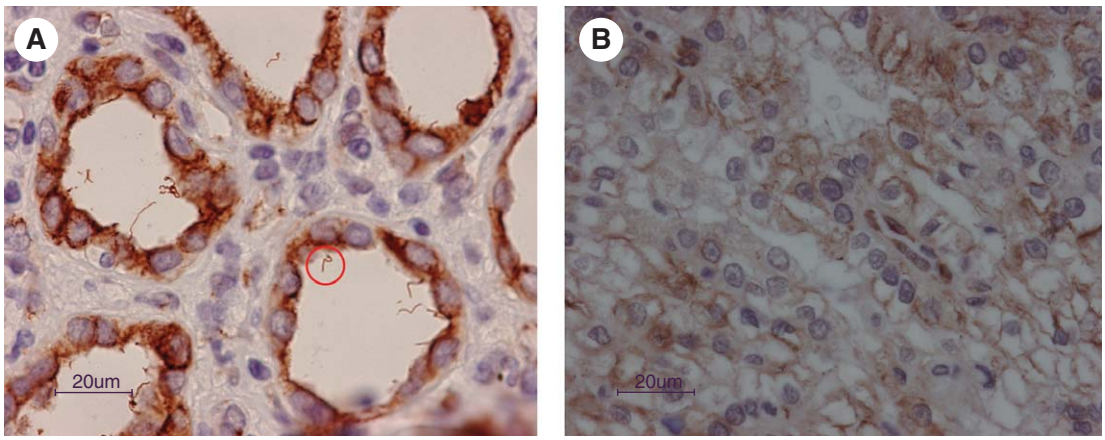


Fig. 3. Acetylated alpha-tubulin stained renal tissue sections. This staining highlights the cells in the walls of the renal tubules and their cilia. (A) Normal parenchyma. Note the organized tubules, regular presence of cilia and the expected lack of staining in the supportive tissue cells between tubules. A primary cilium is encircled. (B) Renal cell cancer parenchyma. Organized tubular structures are lost, staining is diffuse and randomized and noticeable cilia are not present. Note the enlarged alveolar-like appearance of the cells.

veyed incidences of ccRCC implying that, in contrast to mouse models, the loss of pVHL function in humans is a required event in the development of these renal tumors [41]. Another function of the pVHL protein is the regulation of microtubule stability, and the loss of *VHL* has been linked to chromosome segregation errors [53]. Thus, tumor formation could be induced by aneuploidy events independently of cyst formation. These results present the question of whether cilia loss is critical for renal tumorigenesis or if it is just one part of a larger process. Although there is a well-documented proliferation burst in the kidney upon loss of renal cilia, this localized proliferation is not necessarily associated with tumor formation. Polycystic kidney disease has been shown to be caused by ciliary defects but is not associated with tumor formation [58]. Additional signaling pathways are therefore likely to play a role in the transformation of a kidney cyst into a tumor. In the case of RCC, the absence of functional pVHL, the overexpression of survival and proliferative genes as well as the destabilization of microtubules and cilia, combine to contribute to tumor formation. Thus, ccRCC is a good example of the heterogeneity inherent in the function of many ciliary proteins and how the multiple roles of these proteins have complex implications on human diseases.

### 4.2. APC

*APC* is a gene mutated in disease spectrum that share similar diagnoses and manifestations, including familial adenomatous polyposis, attenuated familial adenomatous polyposis, Gardner syndrome, and Turcot syndrome (OMIM 175100 for all). These conditions are characterized by the development of thousands of precancerous colonic polyps which can develop in patients as young as seven with an average age of incidence at 16 yr. In addition, patients may develop osteomas and soft-tissue tumors (Gardner syndrome) or tumors of the central nervous system (Turcot syndrome). For all *APC*-associated conditions, colon cancer is a certainty later in life without colectomy. All of these syndromes are caused by mutations in the *APC* gene and the differences in their symptoms are believed to be modulated by the particular pathogenic variant of mutation. Much like VHL syndrome, *APC* mutations are inherited in an AD manner and 80% of patients also have an affected parent. Molecular diagnosis can establish the basis of disease in upwards of 90% of cases by identifying the causal mutation. Clinical diagnosis is based on the presence of many (100+) colonic adenomatous polyps [59].

The APC protein is also the tumor suppressor protein most frequently mutated in sporadic colorectal cancer [57]. It acts upstream of pVHL [60] and, like pVHL, binds kinesin-2 and regulates microtubule [61–63] and chromosomal stability [64, 65]. Furthermore, inactivation of *Apc* in mouse kidneys results in renal cysts [66], suggesting a role for cilia regulation in neoplastic lesions occurring upon loss of *APC*. While the mechanisms leading to colorectal cancer are unlikely to be cilia-driven, particularly because under physiological conditions enterocytes continuously progress through the cell cycle and refrain from ciliogenesis, some of the syndromic extra-intestinal manifestations of *APC* inactivation can be attributed to ciliary malfunction, such as skin cysts, and congenital hypertrophy of the retinal pigmented epithelium observed in Gardner syndrome patients, which are clinically similar to symptoms occurring in classic ciliopathies [67].

### 4.3. TSC

TSC is a genetic disease associated with benign tumors in the brain, kidneys, heart, eyes, lungs, and skin with an incidence of 1:6,000. TSC usually affects the central nervous system and results in a combination of symptoms including seizures, developmental delay, behavioral problems, skin abnormalities, and kidney disease. Most tumors in TSC patients are not malignant; however, those that are malignant primarily affect the kidneys. Renal cysts and angiomyolipomas occur in >70% of TSC patients between the age of 15 and 30 yr. Prenatal fetus ultrasounds can identify cardiac rhabdomyoma in the hearts of infants but these can also develop in young children with TSC as well. Beyond tumors, TSC patients can manifest seizures, mental retardation, behavior problems, and skin abnormalities. Many TSC patients are diagnosed in the first year of life, and infants with TSC may manifest cardiac rhabdomyomas or seizures at birth. However, clinical features can be subtle initially, or take years to develop. Consequently, TSC can be unrecognized or misdiagnosed for years. Further complicating diagnosis, patients are typically the first in their family and bear de novo heterozygous germline mutations in either *TSC1* or *TSC2* genes, encoding harmarin and tuberin, respectively [68].

In contrast to *VHL*, loss of *TSC1* or *TSC2* in TSC patients increases cilia length in fibroblasts and the

renal tubule [69–71]. Low nutrient levels are thought to increase cilia length to potentiate a delay in cell cycle progression and protect the cell from stress. Because TSC1 and TSC2 are major regulators of energy sensing through the mammalian target of rapamycin/ribosomal protein S6 kinase (mTOR/S6) pathway, failure to activate this pathway in TSC could explain the increased cilia length [72]. Recent data also from leptin-deficient mice show that nutrient-sensing also regulates cilia length through Pten and Gsk3β [73]. In addition to cilia length, changes in mTOR signaling might contribute to tumorigenesis. The mTOR pathway is normally responsible for modulating the cellular response to stress. Activation of mTOR signaling leads to downstream activation of genes that lead to cell survival, growth and a change in cellular metabolism so that the cell can survive in a low oxygen and low nutrient environment. Consequently, aberrant mTOR signaling has been implicated in tumorigenesis and research into mTOR inhibitors as therapeutic agents for the treatment of cancer is a crowded field [74]. Germline mutations in *PTEN* also contribute to increased predisposition of RCC in Cowden syndrome (OMIM # 158350) [75], although the cilia have not been examined in these patients. TSC patients are predisposed to develop benign renal angiomyolipoma but only demonstrate a slightly elevated lifetime risk for RCC; however, in cases where RCC does develop in TSC patients, the age of onset is 25 yr earlier in TSC patients as compared to the general population, at an average age of 28 yr [76, 77].

### *4.4. Birt-Hogg-Dubé syndrome (BHD)*

BHD syndrome is an AD disease caused by mutations in the *FLCN* gene on chromosome 17. BHD (incidence 1:200,000) predisposes individuals to RCC (characteristically chromophobe-oncocytoma hybrid tumors, although other subtypes can also occur), cystic disease of the kidney and lung, as well as benign tumors of the hair follicles in the skin, called fibrofolliculomas [78, 79]. Any combination of these three symptoms can occur, although fibrofolliculomas are the most common manifestation, found on the face and upper trunk in over 80% of people with BHD over the age of 40 yr, and can be very similar in appearance to skin tags. Most patients with BHD are not diagnosed until the second or third decade of life. Pulmonary cysts are also common and can contribute to collapsed lung/ pneumothorax. Molecular diagnosis by sequencing the *FLCN* gene detects mutation in 88% of the patients.

The FLCN protein localizes to the centrosome during interphase and the mitotic spindle during mitosis and *FLCN* knockdown in healthy cells has been shown to delay ciliogenesis [80]. BHD patients as well as *Flcn* heterozygous mice develop renal cysts and neoplasms as well as aberrant activation of mTOR signaling associated with loss-of-heterozygosity [81]. As mentioned above, mTOR activation is believed to contribute to tumor growth, suggesting a classic tumor suppressor function for FLCN. In addition, *FLCN* mutations have been shown to lead to activation of the canonical Wnt signaling pathway [80]. The Wnt family of proteins comprise another fundamental signaling pathway which directs cell polarity and cell fate decisions [82]. Aberrant Wnt signaling has also been linked to cancer [83]. Collectively, these data suggest that FLCN has a role in the nutrient/energy-sensing pathway, similar to tumor suppressors TSC1, TSC2, PTEN and LKB1, and that mutations in *FLCN* probably cause tumorigenesis through the activation of pro-survival pathways.

### *4.5. Hedgehog signaling and medulloblastoma*

The hedgehog signaling pathway is a particularly good example of the importance of the primary cilium in cell communication. The *hedgehog* gene was originally found in genetic experiments in *Drosophila melanogaster* where it was shown to have an effect on body patterning. The gene was eventually shown to code for a secreted protein that, once released by a cell, can control the patterning of neighboring cells. Over time, homologs in many other species were discovered, including the mammalian homologs Indian hedgehog, Desert hedgehog and Sonic hedgehog (Shh). It is now known that the members of the hedgehog family of proteins are responsible for cell-cell communication in a diverse, but evolutionarily conserved, family of signaling cascades that control many types of tissue patterning, e.g. limb formation and midline structures in the brain, spinal cord and the thalamus. As an adjunct to their morphogenic function in development, hedgehog family proteins are also responsible for the maintenance of progenitor and stem cell populations in many adult tissues. For example, during brain development, Shh secreted by Purkinje cells in the cerebellum promotes the proliferation of granule cell precursors through the activation of proliferative genes like *v-myc avian myelocytomatosis viral*

*oncogene homolog (MYC)*. In the adult organ, hedgehog signaling is required to maintain homeostasis of the local neural stem cell population. Its function as a regulator of proliferation has led to hedgehog signaling being implicated in a number of cancers including basal cell carcinomas as previously mentioned as well as rhabdomyosarcomas [84].

Both the developmental function and the homeostasis function of hedgehog signaling require the primary cilium. Hedgehog receptors are enriched in the ciliary membrane and proteins involved in the signaling cascade are found in or around the primary cilium. Secreted proteins must travel to the cilium in order to be modified before being released [85]. This means that mutations, which affect cilia function can affect hedgehog signaling without directly interacting with the pathway. This fact has implications in both the mechanisms of diseases involving hedgehog, and possible treatments for these diseases.

One of the cell types whose homeostasis is controlled through the cilia and hedgehog signaling are the granule neuron precursor (GNP) cells, a cell population which can give rise to medulloblastoma. Although rare in adults, medulloblastoma is the most common type of malignant pediatric brain tumor, accounting for 12–25% of all childhood central nervous system tumors [86]. As mentioned above, Shh signaling controls the expansion of the GNP population. Shh signaling elements concentrate in the primary cilium and the cilium is required for proper Shh signaling. Mice that have had the cilia removed from their GNP population via conditional inactivation of the *Kif3a* gene, which encodes for a kinesin-2 subunit necessary for ciliation, show a drastic decrease in Shh signaling and a corresponding decrease in the proliferation of their GNPs, leading to improper brain development. Removing *Smoothened (Smo)*, a gene encoding for an essential transducer of Shh, lead to the same mouse phenotypes [87]. Conversely, constitutive activation of the Shh pathway in these cells can lead to excessive proliferation and the formation of medulloblastoma. Mice with constitutively active Smo developed medulloblastoma by postnatal day 10 and Smo was found enriched in the primary cilium in these tumors. Intriguingly, by removing *Kif3a* or *Ift88* and therefore blocking ciliogenesis, tumor formation due to excessive Smo signaling was completely blocked. On the other hand, overexpression of Gli2, a transcriptional activator downstream of Smo, did not predispose to medulloblastoma. In fact, tumors were not formed in this experiment until cilia were ablated [15]. Thus, cilia act as both promoters and repressors of tumorigenesis in the same signaling cascade. Although human medulloblastoma caused by activating mutations in *SMO* are rare and no tumors caused by activating *GLI2* mutations along with another mutation leading to a loss of ciliation have ever been found, mutations in many genes that encode for proteins in the Shh signaling cascade and the related Wnt signaling pathways have been seen in tumors taken from patients with primary medulloblastomas [88]. Medulloblastomas taken from sporadic patients have also been observed to have patterns in their ciliation depending on their genetic background [15]. An examination of tumor cilia might therefore be a useful diagnostic tool used to determine the molecular basis of tumorigenesis in patients.

### *4.6. Tumors from undifferentiated embryonic tissue*

An interesting pattern that has emerged in the study of hereditary cancer syndromes involving the primary cilia is the recurrence in many of these syndromes of tumors developing from undifferentiated embryonic tissue as well as precursor cell populations in early life. In addition to RCC, VHL patients develop characteristic tumors in two tissue types derived from embryonic mesonephric tissue: the broad ligament and epididymis [89]. The angiomyolipomas that develop in the kidneys of TSC patients are partially derived from immature smooth muscle cells and the tumors in medulloblastoma are derived from a precursor cell population as mentioned above. In addition to syndromes already discussed, patients with mutations in the dynein arm assembly factor *DNAAF1* develop tumors in the seminiferous vesicle early in life culminating in the accumulation of the testicular germ cell tumor subtype seminoma. DNAAF1 is found in the primary cilium of early germ cells and tumor sections taken from these patients show reduced ciliary frequency, implying a hypothetical role for DNAAF1 and the primary cilium in the normal growth and maintenance of the germ cell population [90]. In a study of 23 Wilms' tumors, another type of pediatric kidney tumor that is partially derived from immature cells, only eight were found to be ciliated when examined with electron microscopy [91]. The fact that many tumors that are characterized by low cilia counts arise from undifferentiated or immature cell populations might imply that

these cells are somehow more sensitive to tumorigenesis caused by misregulation of the primary cilium. Perhaps the tendency towards tumors in these cell types can be accounted for by their reliance on growth and tissue patterning pathways that require the presence of the cilium such as the Shh and Wnt signaling pathways.

## 5. Conclusions and future perspectives

Although the role of ciliary dysfunction in tumorigenesis is far from clear at the moment, certain patterns in the data imply a common set of mechanisms by which tumors arise. It is still unclear if misregulation of cilia causes cell cycle progression or if cell cycle progression and the loss of cilia are related processes controlled by a common upstream factor. However, from the currently available data, we propose a hypothetical model of cilia-related tumorigenesis as follows: a mutation arises or is inherited which affects cilia directly or affects a pathway associated with cilium function. The loss of proper ciliary function contributes to increased proliferation based on both the direct relationship of the cilium with cell division and the absence of proper signaling that requires functional cilia. In some cases, this increase in proliferation may lead immediately to tumor formation depending on the sensitivity of the cell type to ciliary signaling, whether it is embryonic tissue, and the exact function of the mutated gene. In other cases, increased proliferation may lead only to benign growths, which may constitute an unstable pre-cancerous environment that predisposes the cells to further mutations that will result in tumor formation.

A significant amount of further research is needed before this hypothetical model can be validated. The study of cilia is still relatively young, and much more data remains to be gathered. In particular, examination of the primary cilium (% cells that are ciliated, ciliary length, structure) in many well-studied tumor types is still needed. Integrating our understanding of the primary cilium into our understanding of these diseases and *vice versa* will provide a much deeper understanding of the biology of both cancer and the cilium. Nevertheless, these results show the complexity and importance of the primary cilium as an organelle. To what extent cancer can be considered a ciliopathy remains to be seen but therapeutics which target the cilium have the potential to treat many different types of patients. Because of the intersection between cancer and ciliopathies, treatment modifications from both fields have the potential to effectively treat both types of patients. In addition, rare inherited tumor syndromes have a relatively large window for therapeutic intervention, although such treatment might have to be lifelong. Treatments which involve the cilium or ciliary signaling pathways are already being researched, including PTC124 (ataluren) which can allow transcription of proteins despite premature stop codon mutations for the treatment of multiple diseases including Duchenne muscular dystrophy [92, 93]. There are also new possibilities for immunotherapy such as anti-PD-L1 therapy, which activates the immune system to more aggressively seek out cancer cells [94]. However, immunotherapy may not be the best choice for the treatment of hereditary genetic syndromes such as the ones outlined above, given that this may cause a widespread immune response due to the potentially thousands of PD-L1-positive lesions in a single individual. Nevertheless, treatments like these show the exciting potential of the primary cilium as an avenue of future study into the treatment and mechanisms of cancer and other diseases.

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## References

[1] Goetz SC, Anderson KV. The primary cilium: A signalling centre during vertebrate development. Nat Rev Genet 2010;11(5):331-44.

[2] Chaki M, Airik R, Ghosh AK, Giles RH, Chen R, Slaats GG, et al. Exome capture reveals ZNF423 and CEP164 mutations, linking renal ciliopathies to DNA damage response signaling. Cell 2012;150(3):533-48.

[3] Choi HJ, Lin JR, Vannier JB, Slaats GG, Kile AC, Paulsen RD, et al. NEK8 links the ATR-regulated replication stress response and S phase CDK activity to renal ciliopathies. Mol Cell 2013;51(4):423-39.

[4] Airik R, Slaats GG, Guo Z, Weiss AC, Khan N, Ghosh A, et al. Renal-retinal ciliopathy gene sdccag8 regulates dna damage response signaling. J Am Soc Nephrol 2014 (in press).

[5] Barker AR, Thomas R, Dawe HR. Meckel-Gruber syndrome and the role of primary cilia in kidney, skeleton, and central nervous system development. Organogenesis 2014;10(1): 6-107.

[6] Reiter JF, Blacque OE, Leroux MR. The base of the cilium: Roles for transition fibres and the transition zone in ciliary formation, maintenance and compartmentalization. EMBO Rep 2012;13(7):608-18.

[7] Waters AM, Beales PL. Ciliopathies: An expanding disease spectrum. Pediatr Nephrol 2011;26(7):1039-56.
[8] Ellisen LW. Regulation of gene expression by WT1 in development and tumorigenesis. Int J Hematol 2002;76(2):110-6.
[9] Niaudet P, Gubler MC. WT1 and glomerular diseases. Pediatr Nephrol 2006;21(11):1653-60.
[10] Basten SG, Giles RH. Functional aspects of primary cilia in signaling, cell cycle and tumorigenesis. Cilia 2013;2(1):6.
[11] 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):2429-35.
[12] Kim S, Dynlacht BD. Assembling a primary cilium. Curr Opin Cell Biol 2013;25(4):506-11.
[13] Fry AM, Leaper MJ, Bayliss R. The primary cilium: Guardian of organ development and homeostasis. Organogenesis 2014;10(1):62-8.
[14] Yuan S, Sun Z. Expanding horizons: Ciliary proteins reach beyond cilia. Annu Rev Genet 2013;47(1):353-76.
[15] 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):1062-5.
[16] Wong SY, Seol AD, So PL, Ermilov AN, Bichakjian CK, Epstein EH, et al. Primary cilia can both mediate and suppress Hedgehog pathway-dependent tumorigenesis. Nat Med 2009;15(9):1055-61.
[17] Barakat MT, Humke EW, Scott MP. Kif3a is necessary for initiation and maintenance of medulloblastoma. Carcinogenesis 2013;34(6):1382-92.
[18] Han YG, Alvarez-Buylla A. Role of primary cilia in brain development and cancer. Curr Opin Neurobiol 2010;20(1):8-67.
[19] Kim J, Dabiri S, Seeley ES. Primary cilium depletion typifies cutaneous melanoma *in situ* and malignant melanoma PLoS One 2011;6(11):e27410.
[20] Basten SG, Willekers S, Vermaat JS, Slaats GG, Voest EE, van Diest PJ, et al. Reduced cilia frequencies in human renal cell carcinomas versus neighboring parenchymal tissue. Cilia 2013;2(1):2.
[21] Seeley ES, Carriere 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):422-30.
[22] Schraml P, Frew IJ, Thoma CR, Boysen G, Struckmann K, Krek W, et al. Sporadic clear cell renal cell carcinoma but not the papillary type is characterized by severely reduced frequency of primary cilia. Mod Pathol 2008;22(1):31-6.
[23] Gradilone SA, Radtke BN, Bogert PS, Huang BQ, Gajdos GB, LaRusso NF. HDAC6 inhibition restores ciliary expression and decreases tumor growth. Cancer Res 2013;73(7):259-70.
[24] Egeberg DL, Lethan M, Manguso R, Schneider L, Awan A, Jorgensen TS, et al. Primary cilia and aberrant cell signaling in epithelial ovarian cancer. Cilia 2012;1(1):15.
[25] 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.
[26] Yuan K, Frolova N, Xie Y, Wang D, Cook L, Kwon YJ, 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):857-70.
[27] Nobutani K, Shimono Y, Yoshida M, Mizutani K, Minami A, Kono S, et al. Absence of primary cilia in cell cycle-arrested human breast cancer cells. Genes Cells 2014;19(2):141-52.
[28] McIntyre JC, Davis EE, Joiner A, Williams CL, Tsai IC, Jenkins PM, et al. Gene therapy rescues cilia defects and restores olfactory function in a mammalian ciliopathy model. Nat Med 2012;18(9):1423-8.
[29] Zhang Q, Davenport JR, Croyle MJ, Haycraft CJ, Yoder BK. Disruption of IFT results in both exocrine and endocrine abnormalities in the pancreas of Tg737orpk mutant mice. Lab Invest 2004;85(1):45-64.
[30] Robert A, Margall-Ducos G, Guidotti JE, Bregerie O, Celati C, Brechot C, et al. The intraflagellar transport component IFT88/polaris is a centrosomal protein regulating G1-S transition in non-ciliated cells. J Cell Sci 2007;120(Pt 4):628-37.
[31] Mans DA, Vermaat JS, Weijts BG, van Rooijen E, van Reeuwijk J, Boldt K, et al. Regulation of E2F1 by the von Hippel-Lindau tumour suppressor protein predicts survival in renal cell cancer patients. J Pathol 2013;231(1):117-29.
[32] van Dam TJ, Wheway G, Slaats GG, Group SS, Huynen MA, Giles RH. The SYSCILIA gold standard (SCGSv1) of known ciliary components and its applications within a systems biology consortium. Cilia 2013;2(1):7.
[33] Gascue C, Katsanis N, Badano JL. Cystic diseases of the kidney: Ciliary dysfunction and cystogenic mechanisms. Pediatr Nephrol 2011;26(8):1181-95.
[34] Foy RL, Chitalia VC, Panchenko MV, Zeng L, Lopez D, Lee JW, et al. Polycystin-1 regulates the stability and ubiquitination of transcription factor Jade-1. Hum Mol Genet 2012;21(26):5456-71.
[35] Holland AJ, Cleveland DW. Losing balance: The origin and impact of aneuploidy in cancer. EMBO Rep 2012;13(6):501-14.
[36] Holland AJ, Lan W, Cleveland DW. Centriole duplication: A lesson in self-control. Cell Cycle 2010;9(14):2731-6.
[37] Habedanck R, Stierhof YD, Wilkinson CJ, Nigg EA. The Polo kinase Plk4 functions in centriole duplication. Nat Cell Biol 2005;7(11):1140-6.
[38] Maher ER. Genetics of familial renal cancers. Nephron Exp Nephrol 2011;118(1):e21-6.
[39] Berger AH, Knudson AG, Pandolfi PP. A continuum model for tumour suppression. Nature 2011;476(7359):163-9.
[40] Frantzen C, Links TP, Giles RH. Von Hippel-Lindau disease. In: Pagon RA, Adam MP, Ardinger HH, Bird TD, Dolan CR, Fong CT, Smith RJH, Stephens K, editors. GeneReviews. Seattle (WA), 1993-2014. Available at: http://www.ncbi.nlm.nih.gov/books/NBK1463/. Accessed 20th June 2014.
[41] Gerlinger M, Horswell S, Larkin J, Rowan AJ, Salm MP, Varela I, et al. Genomic architecture and evolution of clear cell renal cell carcinomas defined by multiregion sequencing. Nat Genet 2014;46(3):225-33.
[42] Baldewijns MM, van Vlodrop IJ, Vermeulen PB, Soetekouw PM, van Engeland M, de Bruine AP. VHL and HIF signalling in renal cell carcinogenesis. J Pathol 2010;221(2):125-38.
[43] Lolkema MP, Mans DA, Ulfman LH, Volpi S, Voest EE, Giles RH. Allele-specific regulation of primary cilia function by the von Hippel-Lindau tumor suppressor. Eur J Hum Genet 2008;16(1):73-8.
[44] Thoma CR, Frew IJ, Hoerner CR, Montani M, Moch H, Krek W. pVHL and GSK3 beta are components of a pri-

mary cilium-maintenance signalling network. Nat Cell Biol 2007;9(5):588-95.
[45] Schermer B, Ghenoiu C, Bartram M, Muller RU, Kotsis F, Hohne M, et al. The von Hippel-Lindau tumor suppressor protein controls ciliogenesis by orienting microtubule growth. J Cell Biol 2006;175(4):547-54.
[46] Lutz MS, Burk RD. Primary cilium formation requires von hippel-lindau gene function in renal-derived cells. Cancer Res 2006;66(14):6903-7.
[47] Esteban MA, Harten SK, Tran MG, Maxwell PH. Formation of primary cilia in the renal epithelium is regulated by the von Hippel-Lindau tumor suppressor protein. J Am Soc Nephrol 2006;17(7):1801-6.
[48] Troilo A, Alexander I, Muehl S, Jaramillo D, Knobeloch KP, Krek W. HIF1α deubiquitination by USP8 is essential for ciliogenesis in normoxia. EMBO Rep 2013;15(1): 77-85.
[49] Mans DA, Lolkema MP, van Beest M, Daenen LG, Voest EE, Giles RH. Mobility of the von Hippel-Lindau tumour suppressor protein is regulated by kinesin-2. Exp Cell Res 2008;314(6):1229-36.
[50] Lolkema MP, Mans DA, Snijckers CM, van Noort M, van Beest M, Voest EE, et al. The von Hippel-Lindau tumour suppressor interacts with microtubules through kinesin-2. FEBS Lett 2007;581(24):4571-6.
[51] Frew IJ, Smole Z, Thoma CR, Krek W. Genetic deletion of the long isoform of the von Hippel-Lindau tumour suppressor gene product alters microtubule dynamics. Eur J Cancer 2013;49(10):2433-40.
[52] Thoma CR, Matov A, Gutbrodt KL, Hoerner CR, Smole Z, Krek W, et al. Quantitative image analysis identifies pVHL as a key regulator of microtubule dynamic instability. J Cell Biol 2010;190(6):991-1003.
[53] Hergovich A, Lisztwan J, Barry R, Ballschmieter P, Krek W. Regulation of microtubule stability by the von Hippel-Lindau tumour suppressor protein pVHL. Nat Cell Biol 2003;5(1): 4-70.
[54] Hergovich A, Lisztwan J, Thoma CR, Wirbelauer C, Barry RE, Krek W. Priming-dependent phosphorylation and regulation of the tumor suppressor pVHL by glycogen synthase kinase 3. Mol Cell Biol 2006;26(15):5784-96.
[55] Albers J, Rajski M, Schonenberger D, Harlander S, Schraml P, von Teichman A, et al. Combined mutation of Vhl and Trp53 causes renal cysts and tumours in mice. EMBO Mol Med 2013;5(6):949-64.
[56] Frew IJ, Thoma CR, Georgiev S, Minola A, Hitz M, Montani M, et al. pVHL and PTEN tumour suppressor proteins cooperatively suppress kidney cyst formation. EMBO J 2008;27(12):1747-57.
[57] Giles RH, van Es JH, Clevers H. Caught up in a Wnt storm: Wnt signaling in cancer. Biochim Biophys Acta 2003;1653(1):1-24.
[58] Deane JA, Ricardo SD. Polycystic kidney disease and the renal cilium. Nephrology (Carlton) 2007;12(6):559-64.
[59] Jasperson KW, Burt RW. APC-associated polyposis conditions. In: Pagon RA, Adam MP, Ardinger HH, Bird TD, Dolan CR, Fong CT, Smith RJH, Stephens K, editors. GeneReviews. Seattle (WA), 1993-2014. Available at:http://www.ncbi.nlm.nih.gov/books/NBK1345/. Accessed 20th June 2014.
[60] Giles RH, Lolkema MP, Snijckers CM, Belderbos M, van der Groep P, Mans DA, et al. Interplay between VHL/HIF1 alpha and Wnt/beta-catenin pathways during colorectal tumorigenesis. Oncogene 2006;25(21):3065-70.
[61] Jimbo T, Kawasaki Y, Koyama R, Sato R, Takada S, Haraguchi K, et al. Identification of a link between the tumour suppressor APC and the kinesin superfamily. Nat Cell Biol 2002;4(4):323-7.
[62] Kroboth K, Newton IP, Kita K, Dikovskaya D, Zumbrunn J, Waterman-Storer CM, et al. Lack of adenomatous polyposis coli protein correlates with a decrease in cell migration and overall changes in microtubule stability. Mol Biol Cell 2007;18(3):910-8.
[63] Mogensen MM, Tucker JB, Mackie JB, Prescott AR, Nathke IS. The adenomatous polyposis coli protein unambiguously localizes to microtubule plus ends and is involved in establishing parallel arrays of microtubule bundles in highly polarized epithelial cells. J Cell Biol 2002;157(6): 1041-8.
[64] Fodde R, Kuipers J, Rosenberg C, Smits R, Kielman M, Gaspar C, et al. Mutations in the APC tumour suppressor gene cause chromosomal instability. Nat Cell Biol 2001;3(4): 433-8.
[65] Giles RH, Voest EE. Tumor suppressors APC and VHL: Gatekeepers of the intestine and kidney. In: Macieira-Coelho A, editor. Developmental biology of neoplastic growth. Berlin Heidelberg: Springer, 2005; p. 151-181.
[66] Qian CN, Knol J, Igarashi P, Lin F, Zylstra U, Teh BT, et al. Cystic renal neoplasia following conditional inactivation of apc in mouse renal tubular epithelium. J Biol Chem 2005;280(5):3938-45.
[67] Gómez García EB, Knoers NV. Gardner's syndrome (familial adenomatous polyposis): A cilia-related disorder. Lancet Oncol 2009;10(7):727-35.
[68] Northrup H, Koenig MK, Au KS. Tuberous sclerosis complex. In: Pagon RA, Adam MP, Ardinger HH, Bird TD, Dolan CR, Fong CT, Smith RJH, Stephens K, editors. GeneReviews. Seattle (WA), 1993-2014. Available at: http://www.ncbi.nlm.nih.gov/books/NBK1220/. Accessed 20th June 2014.
[69] Armour EA, Carson RP, Ess KC. Cystogenesis and elongated primary cilia in Tsc1-deficient distal convoluted tubules. Am J Physiol Renal Physiol 2012;303(4):F584-92.
[70] DiBella LM, Park A, Sun Z. Zebrafish Tsc1 reveals functional interactions between the cilium and the TOR pathway. Hum Mol Genet 2009;18(4):595-606.
[71] Hartman TR, Liu D, Zilfou JT, Robb V, Morrison T, Watnick T, et al. The tuberous sclerosis proteins regulate formation of the primary cilium via a rapamycin-insensitive and polycystin 1-independent pathway. Hum Mol Genet 2009;18(1): 151-63.
[72] Yuan S, Li J, Diener DR, Choma MA, Rosenbaum JL, Sun Z. Target-of-rapamycin complex 1 (Torc1) signaling modulates cilia size and function through protein synthesis regulation. Proc Natl Acad Sci U S A 2012;109(6):2021-6.
[73] Han YM, Kang GM, Byun K, Ko HW, Kim J, Shin MS, et al. Leptin-promoted cilia assembly is critical for normal energy balance. J Clin Invest 2014;124(5):2193-7.
[74] Porta C, Paglino C, Mosca A. Targeting PI3K/Akt/mTOR signaling in cancer. Front Oncol 2014;4:64.
[75] Shuch B, Ricketts CJ, Vocke CD, Komiya T, Middelton LA, Kauffman EC, et al. Germline PTEN mutation Cowden syndrome: An underappreciated form of hereditary kidney cancer. J Urol 2013;190(6):1990-8.

[76] Crino PB, Nathanson KL, Henske EP. The tuberous sclerosis complex. N Engl J Med 2006;355(13):1345-56.
[77] Henske EP. Tuberous sclerosis and the kidney: From mesenchyme to epithelium, and beyond. Pediatr Nephrol 2005;20(7):854-7.
[78] Toro JR. Birt-Hogg-Dube syndrome. In: Pagon RA, Adam MP, Ardinger HH, Bird TD, Dolan CR, Fong CT, Smith RJH, Stephens K, editors. GeneReviews. Seattle (WA), 1993-2014. Available at: http://www.ncbi.nlm.nih.gov/books/NBK1522/. Accessed 20th June 2014.
[79] Menko FH, van Steensel MA, Giraud S, Friis-Hansen L, Richard S, Ungari S, et al. Birt-Hogg-Dube syndrome: Diagnosis and management. Lancet Oncol 2009;10(12):1199-206.
[80] Luijten MN, Basten SG, Claessens T, Vernooij M, Scott CL, Janssen R, et al. Birt-Hogg-Dube syndrome is a novel ciliopathy. Hum Mol Genet 2013;22(21):4383-97.
[81] Hasumi Y, Baba M, Ajima R, Hasumi H, Valera VA, Klein ME, et al. Homozygous loss of BHD causes early embryonic lethality and kidney tumor development with activation of mTORC1 and mTORC2. Proc Natl Acad Sci U S A 2009;106(44):18722-7.
[82] MacDonald BT, Tamai K, He X. Wnt/beta-catenin signaling: Components, mechanisms, and diseases. Dev Cell 2009;17(1):9-26.
[83] Clevers H. Wnt/beta-catenin signaling in development and disease. Cell 2006;127(3):469-80.
[84] Briscoe J, Therond PP. The mechanisms of Hedgehog signalling and its roles in development and disease. Nat Rev Mol Cell Biol 2013;14(7):416-29.
[85] Nozawa YI, Lin C, Chuang PT. Hedgehog signaling from the primary cilium to the nucleus: An emerging picture of ciliary localization, trafficking and transduction. Curr Opin Genet Dev 2013;23(4):429-37.
[86] Bartlett F, Kortmann R, Saran F. Medulloblastoma. Clin Oncol (R Coll Radiol) 2013;25(1):36-45.
[87] Spassky N, Han YG, Aguilar A, Strehl L, Besse L, Laclef C, et al. Primary cilia are required for cerebellar development and Shh-dependent expansion of progenitor pool. Dev Biol 2008;317(1):246-59.
[88] Thompson MC, Fuller C, Hogg TL, Dalton J, Finkelstein D, Lau CC, et al. Genomics identifies medulloblastoma subgroups that are enriched for specific genetic alterations. J Clin Oncol 2006;24(12):1924-31.
[89] Mehta GU, Shively SB, Duong H, Tran M, Moncrief TJ, Smith JH, et al. Progression of epididymal maldevelopment into hamartoma-like neoplasia in VHL disease. Neoplasia 2008;10(10):1146-53.
[90] Basten SG, Davis EE, Gillis AJ, van Rooijen E, Stoop H, Babala N, et al. Mutations in LRRC50 predispose zebrafish and humans to seminomas. PLoS Genet 2013;9(4): e1003384.
[91] Ito J, Johnson WW. Ultrastructure of Wilms' tumor. I. Epithelial cell. J Natl Cancer Inst 1969;42(1):77-99.
[92] Karijolich J, Yu YT. Therapeutic suppression of premature termination codons: Mechanisms and clinical considerations (review). Int J Mol Cell Med 2014;34(2):355-62.
[93] Li M, Andersson-Lendahl M, Sejersen T, Arner A. Muscle dysfunction and structural defects of dystrophin-null sapje mutant zebrafish larvae are rescued by ataluren treatment. FASEB J 2014;28(4):1593-9.
[94] Lines JL, Sempere LF, Broughton T, Wang L, Noelle R. VISTA Is a novel broad-spectrum negative checkpoint regulator for cancer immunotherapy. Cancer Immunol Res 2014;2(6):510-7.