Molecular targets of primary cilia defects in cancer (Review)

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Abstract. Primary cilia are hair-like organelles that are present on the majority of mammalian cells. They are regarded as the regulatory 'hub' of cell functions due to their indispensable roles for several signaling pathways, such as Hh and Wnt pathways. Originally, cilia defects were found to cause a panoply of human diseases commonly referred to as 'ciliopathies'. Evidence is accumulating that cilia defects are involved in the onset and development of cancer. Some proteins that cause cilia defects have been identified as oncogenes in multiple cancer types. Hence, understanding the pathways that cause cilia defects in cancer is of utmost importance for the development of novel cancer therapeutic targets. The present review article provides a critical overview of the molecular targets of primary cilia defects in cancer, and highlights their vast potential as therapeutic targets and novel biomarkers.

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1. Introduction

Cilia are evolutionally conserved cell structures protruding from the cell surface, which are found ubiquitously across species from ancient protozoa to humans. They are usually divided into two categories: Motile cilia and primary cilia, both of which consist of the axoneme, matrix and ciliary membrane (Fig. 1). Unlike motile cilia, primary cilia usually lack the dynein motor proteins that power axonemal beating and are therefore immotile (1). For almost 100 years, cilia were considered as anomalous structures without any function, until they were found to be associated with the onset of multiple syndromes, such as Bardet-Biedl (2), Joubert (3), oral-facial-digital (OFD) (4), and Ellis-van Creveld syndrome (5).

There is abundant evidence to indicate that primary cilia tightly regulate numerous critical signaling pathways, such as the Hh, Wnt and Notch signaling pathways. Therefore, primary cilia are regarded as the regulatory 'hub' of cell functions (6,7). It is worth noting that, apart from basal cell carcinoma (BCC) (8) and medulloblastoma (9), in which tumorigenesis is cilia-dependent, cilia formation is compromised in multiple tumor types, including melanoma (10), pancreatic cancer (11), breast cancer (12), cholangiocarcinoma (CCA) (13), prostate cancer (14), renal cell carcinoma (RCC) (15) and oral squamous cell carcinoma (OSCC) (16). Since hundreds of proteins and numerous critical signaling pathways are regulated by primary cilia, their proper formation is critical for the integrity of signal transduction and various cellular processes (17). Therefore, molecules that cause primary cilia defects may provide novel approaches with which to attenuate tumor progression, which is the focus of the present review.

2. Primary cilia and the cell cycle

Uncontrolled cell proliferation and deregulation of the cell cycle are hallmarks of malignant tumor formation (18). This section mainly provides a description of the mechanisms through which ciliogenesis is closely associated with the cell cycle.

Cilia assembly and cell cycle. Ciliogenesis is an elaborately regulated process that begins when cells exit the mitotic cycle (Fig. 2). Ciliogenesis occurs through two main routes: The classic intracellular pathway, in which the short axoneme extends from the basal body before the latter reaches the cytoplasmic membrane (19); and the alternative pathway, in which the axoneme extends from the basal body only after the latter is anchored to the cytoplasmic membrane (20). In fibroblasts, cilia are assembled through the intracellular pathway. When the cell exits the cell cycle, cytoplasmic vesicles are

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anchored to the distal appendage of the mother centriole and then trigger the remodeling of the mother centriole into the basal body, which is the earliest ciliogenesis event that can be detected (21). The basal body-vesicle complex then migrates to the cell surface and is anchored to the plasma membrane. Under the transport of intraflagellar transport (IFT) complexes, proteins accumulate into cilia through the 'ciliary gate', and cilia gradually bulge from the cell surface. Conversely, in polarized epithelial and multiciliated cells, ciliogenesis occurs through the alternative pathway. The basal body localizes at the center of the apical membrane and interacts directly with the membrane, with cilia assembly occurring entirely in the plasma membrane [further details on the ciliogenic pathways observed have been previously described (22)].

Cilia disassembly and cell cycle. In contrast to cilia assembly, the mechanisms underlying cilia disassembly in normal or pathological conditions remain largely unknown (23). Following the stimulation of mitogens, the scaffolding protein human enhancer of filamentation 1 (HEF1) activates aurora kinase A (AURKA) in the basal body, which in turn phosphorylates histone deacetylase 6 (HDAC6) (24). Phosphorylated HDAC6 enters the cilia to remove the acetylation modification of axonemal microtubules, thus inducing cilia disassembly. However, the mechansims through which HEF1 is upregulated and recruited to the basal body following mitogen stimulation remain unclear. In addition to HEF1, Trichoplein (25) and Pitchfork (26) have also been proven to be activators of AURKA in the G1 and S phases, respectively.

In addition to HDAC6, kinesin family member (KIF)24 and KIF2a are also implicated in the de-polymerization of axonemal microtubules. The activity of KIF24 is enhanced by never-in-mitosis-A-related kinase 2 (NEK2) during the S and G2 phases (27), and KIF2a is activated by polo-like kinase 1 (PLK1) in the G2 and M phases (28). Notably, the regulatory mechanisms of AURKA, NEK2 and PLK1 involved in cilia disassembly are relatively conservative and in chronological order, ensuring the irreversibility of cilia resorption following cell cycle entry.

Since the centriole dually functions as the microtubule-organizing center during mitosis and as the basal body for ciliogenesis, a common hypothesis is that cilia anchor the centriole to the cell membrane, thereby depriving the cell of cycle entry (23). The abnormal presence of cilia has been shown to lead to cell cycle arrest observed in *in vivo* and *in vitro* models. Trichoplein or AURKA knockdown induce cilia to fail to disassemble, thus inducing G0/G1 arrest. More notably, this phenotype was reversed when cilia formation was prevented by the simultaneous knockdown of IFT20 (25). In a previous study, NudE neurodevelopment protein 1, localizing on the mother centriole, was found to be highly expressed during mitosis and then rapidly degraded when the cell became quiescent. Its silencing in zebrafish and cultured cells led to a significant increase in ciliary length and S phase arrest (29). In addition, in another study, Tctex1 deletion, mainly localizing on the transition zone and regulating ciliary resorption, was shown to resulted in cilia persistence and G1/S phase arrest (30). These studies suggest that cells may sense the presence of primary cilia to limit cell cycle entry; however, further research is required to prove this hypothesis.

Ciliogenesis is a cell cycle-regulated event in normal cells. However, few studies have closely implicated the ciliogenesis changes when the cell cycle is under the influence of malignancies. The most fundamental trait of malignant cells involves their ability to sustain proliferation (31). Therefore, a possible cause of cilia loss in malignant cells could be the increased proliferation. However, Menzl *et al* (12) found that the loss of primary cilia was not associated with an increased proliferative index (Ki67-positive cells) in breast cancer. Nobutani *et al* (32) hardly detected primary cilia in cell cycle-arrested human breast cancer cells. Moreover, in a study involving 110 patients with kidney cancer, reduced ciliary ratio was observed to be independent of cell proliferation (15). It is worth noting that ciliogenesis is not restrained in all malignant tumors. The ciliary ratio has been shown to be significantly increased in BCC tissues (33) and cilia have been identified in some subsets of human medulloblastomas which had activation in either Hh or Wnt signaling (9). These studies suggest that cilia defects are characteristic of oncogenic transformation, and this is not only due to the increased proliferation in malignancies.

3. Molecular targets that cause primary cilia defects in cancer

The abnormal expression of certain critical ciliary proteins, which have a number of the hallmarks of oncogenes, can result in severe cilia defects. Although only a few protein inhibitors have been used in clinical practice or in clinical trials (34), targeting these molecules still holds promise for cancer treatment (35). Nevertheless, a number of studies have been primarily observational and do not elaborate on the mechanisms of cancer containment by targeting these ciliary molecules. This section provides a summary and discussion of the mechanisms through which these molecules can cause cilia defects in cancer (Fig. 3).

Molecules regulating cilia assembly

Cell cycle-related kinase (CCRK). CCRK plays a critical role in promoting G1 phase entry and is of interest as it is the closest vertebrate homolog to the long flagella 2, a critical protein controlling the length of flagella in chlamydomonas (36). CCRK localizes in the cytoplasm and tunes ciliary length and shape by coordinating the assembly of the ciliary axoneme and membrane (37,38). In addition, cell cycle regulation by CCRK has been found to be dependent on primary cilia. The mutation of CCRK in mice induces shortened and swollen cilia, which impairs the Hh pathway and leads to a constellation of developmental defects, including exencephaly, cleft palate and mild preaxial polydactyly/limb skeletal defects (39). Immature cilia with ultrastructural malformation are considered to be a true hallmark of glioblastoma tumors and to be associated with their unrestrained growth (40). Yang *et al* (41) found that the arrested ciliogenesis, and therefore tumor growth, were caused by the elevation of CCRK in glioblastoma. Furthermore, the depletion of *CCRK* markedly increased the ratio and length of primary cilia in glioblastoma cell lines, and notably, cells with restored cilia exhibited a block or severe delay in cell cycle progression, thus inhibiting tumor cell growth (41). It would be of interest to explore the mechanisms of action of CCRK in primary cilia in glioblastoma *in vivo*. On the whole, these

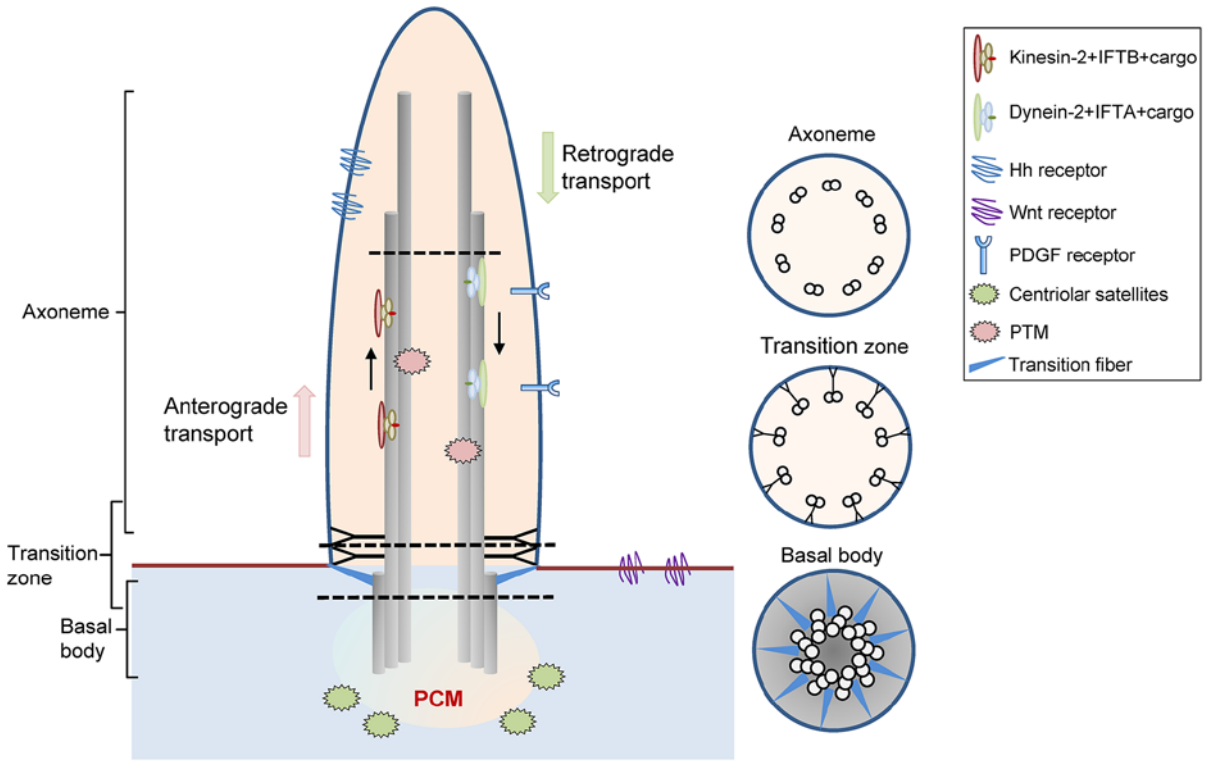

Figure 1. Basic structure of primary cilia. Longitudinal section (left panel) and transverse section (right panel) of primary cilia. The axoneme of primary cilia extends from the basal body, which is anchored to the plasma membrane by transition fibers. Primary cilia contain nine peripheral microtubule pairs and lack a central pair (9+0 pattern), unlike motile cilia (9+2 pattern). Axonemal microtubules are regulated by post-translational modifications. Cargo proteins are transported along the length of the axoneme by IFT, which is powered by kinesin-2 (anterograde transport) or dynein-2 (retrograde transport). IFT, intraflagellar transport; PCM, pericentriolar material; PDGF, platelet-derived growth factor; PTM, post-translational modifications.

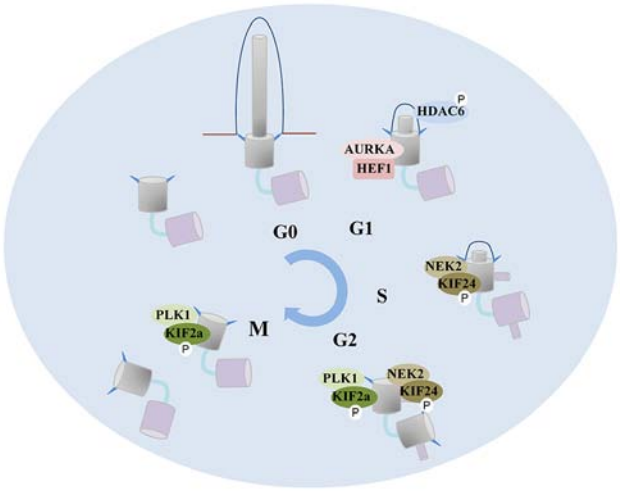

Figure 2. Linkage of primary cilia and centrosomes to the cell cycle. Primary cilia begin to disassemble when cells enter into the cell cycle. The axoneme is depolymerized as cells progress to the S phase, at which centrosomes duplicate and cilia largely disappear. In the early G1 phase, HEF1/AURKA is recruited to the basal body, activating HDAC6. Phosphorylated HDAC6 enters the cilia, de-acetylates microtubules, and leads to axoneme disassembly. At a later point, NEK2 phosphorylating KIF24 and PLK1 phosphorylating KIF2a successively depolymerize the microtubules during the S/G2 and G2/M phases, thereby ensuring that cilia completely disappear. Following mitosis, centrioles are released from the microtubule-organizing center and transform into the basal body again. It is worth noting that only the mother centriole can initiate the assembly process of primary cilia. Narrow gray columns, axonemes; broad grey columns, mother centrioles; purple grey columns, daughter centrioles. AURKA, aurora kinase A; HEF1, human enhancer of filamentation 1; HDAC6, histone deacetylase 6; NEK2, never-in-mitosis-A-related kinase 2; KIF24, kinesin family member 24; PLK1, polo-like kinase 1.

finding indicate that the suppression of primary ciliogenesis by the upregulation of CCRK may be one of the mechanisms used by glioblastoma cells to provide a growth advantage.

PLK4. PLK4 is a conserved centrosomal protein (CEP) that plays a key role in the centriole duplication cycle in a concentration-dependent manner. The centrioles fail to duplicate when PLK4 malfunctions, while they overamplify when PLK4 is overexpressed (42). However, ciliogenesis is blocked, regardless of its deficiency or overexpression. Although abnormalities in centriole number and structure are commonly observed in cancer, cilia defects caused by PLK4 dysfunction in cancer were not reported until 2014. Shinmura *et al* (43) reported that the abnormally elevated expression of PLK4 was related to the absence of primary cilia in human gastric cancer. The upregulation of PLK4 mRNA expression was detected in half of the primary gastric cancers. Moreover, an *in vitro* experiment demonstrated that its overexpression directly caused centrosome amplification, leading to the suppression of ciliogenesis in gastric cancer cell lines (43). Another study overexpressed PLK4 in mice and found that the formation of primary cilia was prevented, cilia signaling was disrupted, and the formation of lymphomas and sarcomas in p53 null mice was advanced (44).

Thymosin β4 (Tβ4). Tβ4, a 43-amino acid peptide (45), is widely distributed in several cell types, apart from red blood cells, and regulates actin cytoskeleton dynamics by sequestering globular actin monomers (46). Its elevation is highly associated with tumor malignancy in multiple cancer types, including melanoma, head and neck squamous cell carcinoma

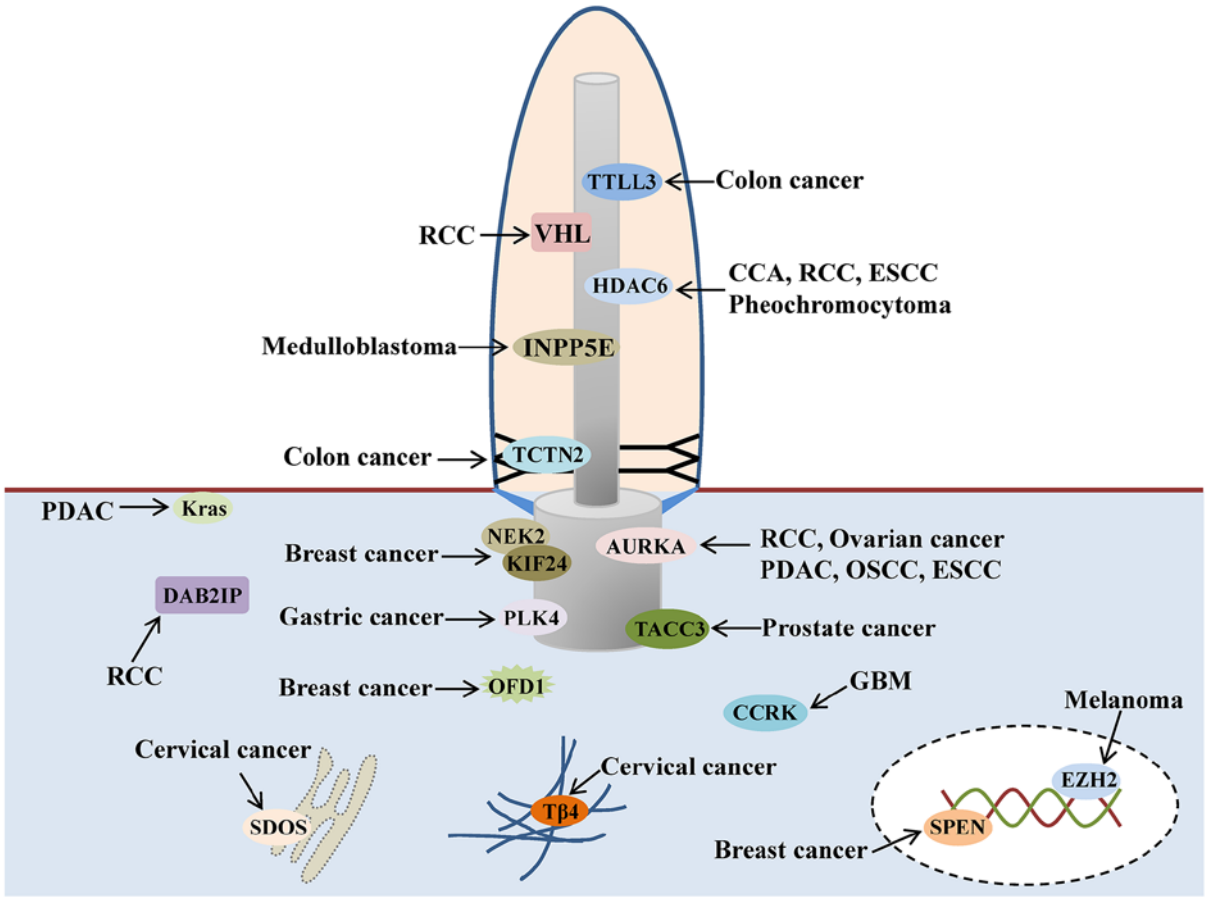

Figure 3. Overview of molecular targets of cilia defects in different tumor types. This schema was drawn according to the subcellular location of molecules when they regulate the ciliary biology. Further details can be found in the manuscript. CCA, cholangiocarcinoma; ESCC, esophageal squamous cell carcinoma; GBM, glioblastoma; OSCC, oral squamous cell carcinoma; PDAC, pancreatic ductal adenocarcinoma; RCC, renal cell carcinoma; VHL, Von Hippel-Lindau; SDOS, syndesmos; DAB2IP, DAB2-interacting protein; TTLL3, tubulin tyrosine ligase like 3; HDAC6, histone deacetylase 6; INPP5E, inositol polyphosphate 5-phosphatase E; TCTN2, tectonic 2; AURKA, aurora kinase A; NEK2, never-in-mitosis-A-related kinase 2; KIF24, kinesin family member 24; TACC3, transforming acidic coiled-coil protein 3; CCRK, cell cycle-related kinase; SPEN, split ends; EZH2, enhancer of zeste homolog 2.

and bladder cancer (47-49). The cilia ratio is increased by Tβ4 overexpression by upregulating the expression of nephronophthisis 3 (localizing to the basal body and indispensable for cilia integrity) in HeLa human cervical cancer cells (50). Further research by the same research group demonstrated that Tβ4-mediated cilia formation may control HeLa cell growth induced by di-(2-ethylhexyl) phthalate, a xenoestrogen with carcinogenic toxicity (51).

Thus far, the presence of cilia defects in cervical cancer remains contradictable and elusive. Primary cilia were rare in HeLa cells in the study by Alieva *et al* (52); however, in the study by Kowal and Falk (53), the ciliated population was significantly higher than previously anticipated. In addition, due to the extensive presence of primary cilia, the HeLa cell line was used as a model to explore cilia biology (54). However, these studies only examined primary cilia in the cultured cells and did not set up a strict control group of normal cervical epithelial cells. Therefore, further research is warranted at the tissue level.

Tectonic (TCTN)2. The TCTN family (including TCTN1, TCTN2 and TCTN3) forms complexes with proteins that localize to the transition zone of cilia, where they regulate the composition of the ciliary membrane in a tissue-dependent manner (55). Mutations in *TCTN*s lead to tissue-specific defects in cilia assembly and trafficking that underlie several ciliopathies (56). However, the available studies on the role of TCTNs in cancer are limited. TCTN1 elevation predicts poor clinical outcomes for patients with glioblastoma (57) and is indispensable for pancreatic cancer cell proliferation (58). Its association with primary cilia in these two cancer types has not yet been investigated, at least to the best of our knowledge. A previous study identified TCTN2 as an oncogene and a tumor marker in several cancer types, including colorectal, lung and ovarian cancer (59). Moreover, inhibiting the expression of TCTN2 has been found to significantly reduce colony formation and cell invasiveness, and impair the assembly of primary cilia in colon cancer cell lines. However, the existing studies are contradictory. Yasar *et al* (60) observed that the cilia number was elevated in colon adenocarcinoma compared with normal tissues, whereas the study by Rocha *et al* (61) demonstrated that the cilia number was decreased in colon cancer.

DAB2-interacting protein (DAB2IP)-KIF3a. DAB2IP, a member of the RasGTPase-activating protein family, has been identified as a tumor suppressor in several cancer types (62,63). Its loss is highly prevalent in different subtypes of RCC and is associated with a poor patient survival (64). A recent study found that DAB2IP knockdown impaired primary cilia formation by decreasing the expression of KIF3a (essential for

cilia assembly and length maintenance) in kidney epithelial cells (65). In addition, the loss of KIF3a further promotes renal tumorigenesis, suggesting that primary cilia stability is part of the critical homeostatic machinery in renal epithelia (65).

Transforming acidic coiled-coil protein 3 (TACC3). TACC3, characterized by a highly conserved C-terminal coil domain, is a key component of centrosome-microtubule dynamic networks (66). Recently, TACC3 has been identified as a potential prognostic marker and therapeutic target for various cancer types, such as breast (67) and lung cancer (68). Cilia ratio and length are gradually decreased during the progression from normal prostate to prostatic intraepithelial neoplasia and invasive prostate cancer, and that increase is accompanied by the activation of Wnt signaling (14). However, the mechanisms responsible for cilia defects in prostate cancer remain elusive. A recently published study demonstrated that TACC3 upregulation restrained ciliogenesis in prostate cancer cells (69). TACC3 can competitively bind filamin A and disrupt the michelin-filamin A interaction, which is necessary for centrosome migration to the apical membrane and cilia formation (70). Furthermore, TACC3 knockdown significantly restored the formation of primary cilia and inhibited tumorigenesis and tumor growth *in vitro* and *in vivo*, suggesting that targeting TACC3 may represent a novel approach for prostate cancer therapeutics (69).

Molecules regulating cilia disassembly

AURKA-HDAC6 signaling axis. As mentioned above, the AURKA-HDAC6 signaling axis plays a critical role in cilia disassembly. AURKA activation is common in multiple cancer types characterized by centrosomal amplification and genomic instability (71,72). The oncogenic AURKA appears to be the key node for the suppression of primary cilia in several types of cancer, including pancreatic ductal adenocarcinoma (PDAC) (73), clear cell RCC (ccRCC) (74), ovarian cancer (75), pheochromocytoma (76) and esophageal squamous cell carcinoma (ESCC) (77). In addition, in a previous study, the number of cilia was significantly decreased in AURKA-activated tissues from patients with OSCC (16). More importantly, AURKA inhibition has been shown to result in primary cilia re-expression and significantly inhibit tumor progression in PDAC, ccRCC and OSCC (16,73,78).

However, the target of AURKA for cilia absorption may vary between tumor types. Unlike ccRCC (74), pheochromocytoma (76) and ESCC (77), cilia loss induced by AURKA activation is independent of HDAC6 in PDAC (73), ovarian cancer (75) and OSCC (16). The specific target of AURKA in these cancer types is unclear. A previous study demonstrated that, in addition to HDAC6, AURKA also activated inositol polyphosphate 5-phosphatase E (INPP5E) (79), a ciliary protein whose absence results in ciliary destabilization and tumor progression in medulloblastoma (80). AURKA may promote cilia loss through INPP5E in PDAC, ovarian cancer and OSCC, which requires further verification.

Gradilone *et al* (81) focused on the mechanisms of HDAC6 on cilia resorption and cell proliferation in CCA. Their study reported a marked reduction in the number of cilia, which was accompanied by HDAC6 overexpression in clinical CCA tissues. Inhibiting HDAC6 by the pharmacologic inhibitor tubastatin-A or by shRNA could re-express primary cilia in CCA cell lines and decrease cell proliferation. More interestingly, the inhibitory effect of tubastatin-A was abolished after ciliogenesis was blocked by *IFT88* knockdown, indicating that the inhibitory effect on cell proliferation through targeting HDAC6 is partially dependent on cilia restoration in CCA cells (81). The latest research from the same research group demonstrated that cilia disassembly in CCA was mediated by HDAC6-regulated autophagy in primary cilia (i.e., ciliophagy), suggesting that ciliophagy inhibition could be an important therapeutic target for CCA (82).

NEK2-KIF24 signaling axis. KIF24, localizing to the distal end of the centriole, depolymerizes the cilia microtubules and provokes cilia disassembly shortly following the stimulation of mitogens (27). The depolymerizing activity of KIF24 is enhanced by NEK2, a kinase only expressed in the S and G2 phases (83). The NEK2-KIF24 action at centriole prevents the aberrant assembly of cilia and keeps the de-ciliated state necessary for mitosis. This mechanism of inhibiting primary ciliogenesis is temporally distinct from the well-established AURKA-HDAC6 pathway by blocking the nucleation of cilia from the basal body (83).

NEK2 has been identified as an oncogene in various cancer types, including myeloma and breast cancer (84-86). Cilia loss is considered to be a characteristic of breast cancer, and the mouse model further confirmed its importance in the development of breast cancer. Although the absence of primary cilia was not found to directly cause breast cancer in mice, it led to the earlier formation and accelerated the growth of cancer, and was associated with a higher grade and metastasis (87). Kim *et al* (83) found that NEK2-KIF24 was overexpressed in breast cancer cell lines, and their ablation resulted in the re-expression of primary cilia, thereby reducing the proliferation of cancer cells. However, NEK2 has not been reported to induce the loss of primary cilia in other tumors apart from breast cancer.

Tubulin tyrosine ligase like 3 (TTLL3). The glutamylation of tubulin in mammals can be catalyzed by nine glutamate ligases, in which only two enzymes, TTLL3 and TTLL8, are capable of initiating glycylation on microtubules (88). Glycylation has been found to function as a critical regulator of ciliary disassembly in motile cilia and flagella, while previous studies that used zebrafish or mouse models proved that the integrity of primary cilia was also dependent on this post-translational modification on tubulin (89,90). TTLL3 is the only expressed glycylase in colon tissue, and its knockout in mice has been shown to not only cause a marked reduction in the number of cilia, but to also lead to a markedly increased cell proliferation rate in the colon epithelium. In addition, the lower expression level of TTLL3 has been shown to be significantly associated with the development of colorectal carcinoma, suggesting that cilia defects caused by TTLL3 may serve as a prognostic marker for this type cancer (61).

Molecules playing dual roles in cilia biology

Tuberous sclerosis complex (TSC)-mTOR complex 1 (mTORC1) pathway. TSC is a genetic syndrome with widespread dysplastic and multisystemic tumors, caused by the mutations in the tumor suppressor genes, TSC1 and TSC2 (91). TSC1 and TSC2 form a heterodimer that inhibits mTOR signaling by inactivating mTORC1, a type of complex

sensitive to rapamycin (92). TSC1, localizing to the basal body (93), is frequently heterozygously lost in ccRCC (94). Activating mTOR signaling by silencing TSC1 lengthened primary cilia in zebrafish (95) and mouse (96) models, and inhibiting mTOR signaling by rapamycin, could return the ciliary length to normal (96). Rosengren *et al* (97) also found that mTORC1 inhibition by rapamycin resulted in shortened cilia. These studies appear to indicate that mTORC1 plays a positive role in ciliary maintenance. However, another study reported the negative effects of mTORC1 on primary cilia, in which ciliary length was increased following rapamycin treatment in a dose-dependent manner in renal epithelial and vascular endothelial cells (98).

Takahashi *et al* (99) found that mTORC1 played dual roles in ciliogenesis, since rapamycin treatment shortened the cilia length, whereas it promoted the cilia ratio in retinal pigmented epithelial (RPE1) cells. Furthermore, rapamycin treatment markedly restored the formation of primary cilia and attenuated cell proliferation in lung, kidney, breast and pancreatic cancer cell lines (99). Although the reasons for these discrepancies are unclear, in renal cancer (100) and patients with polycystic kidney disease (101), primary cilia defects are often accompanied by the aberrant activation of mTORC1. In addition, rapamycin and its derivatives have been widely used in the treatment of advanced renal (102) and breast cancer (103).

OFD1 and autophagy. The *OFD1* gene was initially identified in OFD syndrome (104) and associated with other ciliopathies, such as Joubert syndrome and retinitis pigmentosa (105). As a component of the distal centriole, OFD1 builds centriole distal appendages, recruits IFT88 and stabilizes centriolar microtubules at a defined length to properly assemble cilia (106). Further research identified that OFD1, similar to CEP290 and pericentriolar material 1, is also the primary component of centriolar satellites, the particles surrounding centrosomes (107). Of note, OFD1 at the centriolar satellites functions as a suppressor of primary cilia, the opposite of the promotion of ciliogenesis observed in distal centrioles. Deficient autophagy-induced OFD1 populated centriolar satellites, leading to fewer and shorter primary cilia and, interestingly, partial OFD1 knockdown significantly restored the cilia formation in MCF7 cells, a human breast cancer cell line that completely lacks primary cilia (108).

The aforementioned study indicated a positive role of autophagy in the regulation of primary cilia formation, while another report recognized basal autophagy as a suppressor in ciliogenesis in primary Hürthle cell tumors. Cilia loss was caused by a high basal autophagic flux, and the inhibition of autophagosome formation notably restored the primary ciliogenesis in tumor cells (109). Pampliega *et al* (110) also found that autophagy inhibited ciliogenesis and cilia-associated signaling during normal nutritional conditions. On the other hand, Maharjan *et al* (111) reported that alterations in autophagy during serum-restimulation, irrespective of whether autophagy was activated or inhibited, prevented the disassembly of primary cilia in RPE1 cells. These studies suggest that the regulation of autophagy on primary cilia is likely dependent on the cellular context. Furthermore, the association between primary cilia and autophagy is bidirectional, since the abrogation of ciliogenesis partially inhibits autophagy (110). The regulatory mechanisms between autophagy and primary cilia are further complicated by other cellular events that are usually observed in cancer, such as the activation of AURKA, HDAC6 or other critical factors controlling cilia biology, and thus warrant further investigation (112).

Von Hippel-Lindau (VHL). VHL has been widely accepted as a component of an E3 ubiquitin ligase that targets hypoxia-inducible factor α for ubiquitination and degradation in an oxygen-dependent manner (113). Its mutation results in VHL disease, the most well-known familial kidney cancer syndrome (114). ccRCC is the most common subtype of renal cancer, and is mainly sporadic. Of note, the *VHL* gene is inactivated in up to 87% of sporadic ccRCC cases (115). In addition, the re-expression of VHL protein is sufficient to suppress the formation of renal cancer *in vivo*, suggesting that VHL inactivation is a direct cause of renal tumorigenesis (116).

Primary cilia are almost absent in the renal cysts and ccRCC of patients with VHL (15). In addition, the histological manifestations of early-stage RCC, including increased disorganized cilia, occurred in the kidney of the *VHL* knockout zebrafish (117). However, in a previous study, ccRCC was not induced by a specific deletion of *VHL* in mouse renal epithelial cells, suggesting that additional mutations are required in mammals (118). The combined conditional inactivation of VHL and other tumor suppressor factors, Pten or tumor protein p53, gave rise to cilia ablation, renal cysts and neoplastic growth resembling human ccRCC (119,120). Of note, another study detected the mutations of ciliary genes in ~50% of 448 human ccRCC samples, suggesting that the dysfunction of primary cilia plays an important role in at least part of ccRCC (121).

The formation of primary cilia has been shown to be restored by re-expressing the wild-type VHL in VHL-defective ccRCC cell lines (122). However, the mechanisms responsible for cilia defects caused by VHL mutation remain elusive. Schermer *et al* (116) found that VHL localized on primary cilia and regulated the cilia maintenance by directing the growth of microtubules toward the cell periphery, which is a prerequisite for ciliogenesis. However, a different study revealed that *VHL*-knockdown led to cilia disassembly by upregulating the expression of NEK8, a cell cycle regulator (123). In addition, VHL knockdown increased AURKA expression by activating β-catenin, thus leading to cilia disassembly. Furthermore, the β-catenin responsive transcription inhibitor rescued cilia defects by inhibiting AURKA, opening new avenues for treatment with β-catenin inhibitors to rescue ciliogenesis in ccRCC (74).

Molecule regulating the transcription of ciliary genes

Split ends (Spen). Spen, characterized by N-terminal RNA-binding motifs, is a large nuclear protein and a component of the HDAC corepressor complex (124). Spen regulates the expression of key transcriptional effectors in multiple signaling pathways (125) and has been established as a tumor suppressor gene by negatively regulating the transcription of estrogen receptor α targets in breast cancer (126). Recently, Spen was found to be co-expressed with the ciliogenic transcription factor, regulatory factor X family member 3. The knockdown of Spen considerably inhibited the formation of primary cilia, and its re-expression rescued ciliogenesis in breast cancer cells (127). Furthermore, the regulation of cell migration by Spen only occurred in those cells harboring

primary cilia, indicating that Spen may coordinate cellular movement in a cilia-dependent manner in breast cancer (127).

Enhancer of zeste homolog 2 (EZH2). EZH2, a histone methyltransferase, is part of polycomb repressive complex 2 (PRC2), which silences target genes epigenetically by catalyzing histone H3 tri-methylation (128). EZH2 is activated in a variety of cancer types and drives cancer progression by suppressing the expression of various tumor suppressor genes (129). Cilia loss is considered to be a promoter and a potential biomarker in melanoma development (10). A recent study (130) reported an inverse correlation between primary cilia and EZH2 expression levels during melanoma development. Activated EZH2 was a driver of melanoma oncogenesis by silencing genes related to ciliary integrity and thus deconstructing primary cilia. Cilia deconstruction further led to the activation of the Wnt/β-catenin pathway, a well-known oncogenic signaling pathway in melanoma. Strikingly, EZH2 activity blockage significantly induced primary ciliogenesis and cilia-dependent tumor growth-arrest, suggesting that rescuing ciliogenesis by targeting EZH2 may serve as a new strategy for melanoma treatment (130).

Syndesmos (SDOS). SDOS was initially reported to co-localize with syndecan 4 cytoplasmic domain in focal contacts and promotes the assembly of focal adhesions (131). A recent study identified SDOS as a novel RNA-binding protein that interacted with TNF receptor-associated protein 1 at the endoplasmic reticulum to regulate mRNA translation (132). A small subset of mRNAs responsible for the primary ciliogenesis was regulated post-transcriptionally by SDOS, including transmembrane protein 67, coiled-coil and C2 domain containing 2A and Kif7, known as the ciliopathy-associated genes. Furthermore, the regulatory effect of SDOS on primary cilia was further proven in HeLa cells. The number and length of primary cilia were significantly increased in SDOS-silenced HeLa cells, whereas they were decreased in SDOS-overexpressing cells.

Others

KRAS proto-oncogene, GTPase (KRAS). KRAS, having intrinsic GTPase activity, is a small GTP-binding protein and functions as a molecular switch for various cellular processes (133). Its mutation, a common driver in various cancers, locks the protein into the GTP-bound state and results in constitutive signaling, which gives a growth advantage to mutated cells, thereby leading to the development of cancer (134,135). In PDAC, KRAS is the most commonly mutated gene, which is present in >90% of tumor cells (136). In addition, the activation of the oncogenic KRAS allele directly induced the formation of PDAC in mice and blocked ciliogenesis in cancer cells (11). Of note, cilia defects were rescued by inhibiting KRAS effector pathways. The present study raises the possibility that aberrant KRAS signaling may promote carcinogenesis by inducing cilia loss in PDAC.

4. Conclusions and future perspectives

Primary cilia were previously considered as vestigial organelles, while recent advances have recognized the complex biological functions of these unique structures in diseases and cancer. Abnormal signaling pathways lead to uncontrolled proliferation (137), drug resistance (138) and immune escape in cancer (139). As the 'hub' for multiple signaling receptors and downstream effector molecules, primary cilia are considered to be the critical regulatory center for inducing pathway defects (140). Primary cilia are likely to impact cancer development in multiple ways, including affecting the cell cycle process (83), mediating signal transduction (141) and regulating the response to therapy targeted to ciliary proteins or related pathways (142). Therefore, several studies have highlighted the possible application of cilia dysfunction in the early diagnosis and prognosis pf cancer.

Primary cilia have been recognized as an important therapeutic target, although the mechanisms of cilia defects are variable in the context of each cancer type (Fig. 3). As already aforementioned, several key factors regulating primary cilia, such as AURKA, HDAC6 and NEK2, have been proven to function as critical oncogenes in the development of various cancer types (13,71,122). Targeting these oncogenic factors and rescuing ciliogenesis can restrain tumor growth, particularly when simultaneously targeting multiple proteins that cause cilia defects. Therefore, targeting these molecules to develop novel therapeutic approaches has been an emerging field in the research of pancreatic, lung, kidney and breast cancer (143).

Although a number of researchers have reported that tumor growth was arrested by re-expressing primary cilia *in vivo* and *in vitro*, further extensive research is warranted before targeting cilia can be used in cancer treatment. Firstly, the mechanisms through which primary cilia regulate carcinogenesis differs between cancer types and within cancer subtypes. Secondly, targeting cilia can both restrain tumor progression and induce kinase inhibitor resistance, which appears to prevent achieving good therapeutic effects (142). Primary cilia are complex organelles whose structure, arrangement and function are highly regulated. However, further studies are required to decipher the complex signals they transmit. Nevertheless, existing studies have suggested that the status of primary cilia should play a role in the decision making for precise and personalized treatment.

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Authors' contributions

FY performed the literature search and wrote the manuscript. ZW, CX and FC collected the relevant references and edited the manuscript. QC supervised and revised the manuscript. All authors have read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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