Primary cilia and cancer

Subjects: Oncology

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Primacy cilia are antenna-like structures present in many vertebrate cells. These organelles detect extracellular cues, transduce signals into the cell, and play an essential role in ensuring correct cell proliferation, migration, and differentiation in a spatiotemporal manner. Not surprisingly, dysregulation of primary cilia can cause various diseases, including cancer. The structure and function of primary cilia are dynamically regulated through many proteins and various posttranslational mechanisms of these proteins, including phosphorylation, acetylation, and ubiquitination. Targeting these signaling that regulates the assembly and disassembly of primary cilia may be a promising approach for cancer treatment.

Keywords: primary cilia ; cancer ; proliferation ; ubiquitin ligase ; deubiquitinase ; protein-protein interaction ; post-translational modification ; migration ; differentiation

1. Introduction

Primary cilia are antenna-like structures, 1–10 micrometer in length, that are present in a variety of vertebrate cells [1][2][3][4][5][6][7]. Primary cilia contain receptors and channels that detect signals from extracellular cues, such as mechanical flow and chemical stimulation, and transduce them into the cell, where they contribute to the maintenance of proper development and homeostasis. Considering these functions, it is not surprising that dysregulation of primary cilia function can cause cancer and other diseases, including ciliopathies, which manifest as various disease phenotypes such as congenital anomalies, neurodevelopmental disorders, and obesity [1][6][7][8][9][10].

The structure and function of primary cilia are dynamically and precisely regulated, enabling cells to proliferate, migrate, and differentiate in a spatiotemporally controlled manner [6][7][11]. The primary cilium is composed of three compartments: the basal body, the transition zone, and the axoneme [2]. The basal body is derived from the mother centriole. Both centrioles and basal body contain nine circularly arranged triplets of microtubules (A-, B-, and C-tubules). The axoneme consists of nine microtubule doublets projected from the A- and B-tubules of basal body. In contrast to motile cilia, a central pair of singlet microtubule is absent (+0) in primary cilia [12][13][14][15]. Therefore, the axoneme of primary cilia is described as 9´2+0. The transition zone is a short area located above the basal body characterized by Y-shaped fibers connecting the microtubule doublets to the ciliary membrane [16].

Primary cilia are disassembled and assembled when cells enter mitosis and exit the cell cycle, respectively [17][18][19]. The formation of primary cilia starts with the binding of small cytoplasmic vesicles transported from the Golgi apparatus to the mother centriole and conversion from the mother centriole to the basal body. The basal body is then moved and anchored to the plasma membrane. Coiled-coil protein 110, a component of the inhibitory complex of ciliogenesis, is removed to initiate axoneme elongation [20][21]. The ciliary vesicle then fuses with the plasma membrane, and large amounts of tubulin are transported from the cytoplasm into the cilium to extend the axoneme [22]. Many signaling molecules are also transported from the cytoplasm into the cilium (anterograde) and from the cilium into the cytoplasm (retrograde) by kinesin and dynein, respectively, which are motor proteins that travel along the axoneme [23].

Various types of posttranslational modification, including phosphorylation, acetylation, and ubiquitination, are involved in the dynamic regulation of the structure and function of cilia [2][4][5][6][7][24]. Modification of proteins by attachment of ubiquitin, a highly conserved 76-amino acid protein, is a critical step in targeting the selective degradation of proteins by proteasomes as part of the ubiquitin–proteasome system (UPS) [25]. Protein ubiquitination occurs in three steps. First, ubiquitin-activating enzymes (E1) bind to ubiquitin, which is expressed in all cell types; second, ubiquitin is transferred from E1 enzymes to ubiquitin-conjugating enzymes (E2); and finally, ubiquitin-ligating enzymes (E3) transfer the ubiquitin from E2 enzymes and ligate it to lysine residues on the target protein. To date, 2, approximately 40, and about 600 E1, E2, and E3 enzymes, respectively, have been identified in humans [25]. The selectivity of target protein ubiquitination is conferred by the combination of E2 and E3 enzymes. Protein ubiquitination is counteracted by deubiquitinase (DUB)-mediated removal of ubiquitin moieties from ubiquitinated proteins [26]. About 100 DUBs have been identified in humans. The balance between ubiquitination and deubiquitination of target proteins and their proteasomal degradation are tightly

regulated processes, and dysregulation of the UPS has been detected in various disorders [27][28][29]. Several lines of evidence support a major role for the UPS in regulating the structure and function of cilia [4][5][6][7][30][31][32][33][34], suggest that the proteins involved in the assemble and disassemble of primary cilia through UPS may serve as novel therapeutic targets for the development of treatments for cancer and other disorders related to the dysregulation of primary cilia [7].

2. Roles of primary cilia in cancer

Primary cilia in cultured mouse 3T3 fibroblasts and human retinal pigment epithelial (RPE1) cells can be disassembled and assembled by serum stimulation and deprivation, respectively [17][18][35]. Aurora A kinase (AURKA), one of the most important mitotic kinases for cell-cycle control [36], plays important roles in deciliation by serum stimulation [37][38]. AURKA is activated by serum stimulation through Ca^{2+}/calmodulin signaling, the non-canonical WNT pathway, and phosphatidylinositol signaling [38][39][40][41]. Serum stimulation also activates AURKA through the pathway involving epidermal growth factor receptor (EGFR), ubiquitin specific peptidase 8 (USP8), and trichoplein (TCHP) (described in the next section) [32][33][42]. Activated AURKA phosphorylates itself and target proteins during G1 phase, which stimulates the disassembly of primary cilia [38]. Several proteins associated with AURKA and ciliogenesis have been identified, including histone deacetylase 6 [35] and nudE neurodevelopment protein 1 (NDE1) [43]. In response to serum stimulation, NDE1 localizes at the basal body and suppresses ciliogenesis by tethering dynein light chain 1 [44]. Under serum deprivation conditions, cyclin-dependent kinase 5 is activated and phosphorylates NDE1. Phosphorylated NDE1 is then recognized and ubiquitylated by the E3 ligase complex SCF^{FBXW7}, resulting in ciliogenesis [45][46]. Importantly, forced ciliation in cells growing under serum stimulation conditions can cause cell-cycle arrest [32][33][42][43][47]. These findings suggest that the primary cilium can act as a negative regulator of the cell cycle and may be a tumor suppressor organelle [3][4][5][6][7][10][48][49][50]. In fact, suppression of primary cilia function is associated with tumorigenesis, cell proliferation, and metastasis in many cancers, including glioblastoma [51], esophageal cancer [52], colon cancer [53], cholangiocarcinoma [54][55], pancreatic ductal adenocarcinoma [56], clear cell renal carcinoma [57], prostate cancer [58], ovarian cancer [59][60], melanoma [61], and chondrosarcoma [62] (**Table 1**). However, primary cilia can promote tumor progression under certain conditions. In medulloblastoma and basal cell skin carcinoma caused by gain-of-function mutation of SMO, primary cilia convert the GLI transcription factors GLI2 and GLI3 to their activated forms, inducing their translocation to the nucleus, increased transcription of Hedgehog target genes, and promotion of cell proliferation [63][64]. In contrast, primary cilia of medulloblastoma and basal cell skin carcinoma caused by gain-of-function mutation of GLI2 increases the activity of GLI3 as a transcriptional repressor, resulting in suppression of proliferation of these cancer cells [63][64]. Further work will thus be necessary to fully understand the context-dependent roles of primary cilia in cell proliferation.

Table 1. The roles of primary cilia in cancer.

Cancer cell	The role of primary cilia (PC) in the cancer	References
Glioblastoma	Inhibition of HDAC6 restores the loss of PC and suppressed the proliferation.	[51]
Esophageal squamous cell carcinoma	Knockdown (KD) of PRDX1 restores the loss of PC and suppressed the proliferation.	[52]
Colon cancer	Knockout of TTLL3 causes the loss of PC and promotes tumorigenesis in colon.	[53]
Cholangiocarcinoma	The number of PC is frequently reduced. Inhibition of HDAC6 restores the loss of PC and suppressed the proliferation	[54][55]
Pancreatic ductal adenocarcinoma	Inhibition of HDAC2 in Panc1 induces ciliogenesis and suppressed the proliferation.	[56]

Clear cell renal carcinoma	PC is lost by inactivation of VHL tumor suppressor.	[57]
Prostate cancer	KD of TACC3 restores the loss of PC and suppressed the proliferation.	[58]
Epithelial ovarian cancer	The number of PC is reduced, which is associated with centrosomal localization of AURKA. KD of AURKA restores the loss of PC and suppressed the oncogenic hedgehog signaling.	[59][60]
Melanoma	Deconstruction of PC is sufficient to drive metastatic formation.	[60]
Chondrosarcoma	Inhibition of HDAC6 restores the loss of PC and suppressed the proliferation.	[62]
Medulloblastoma, basal cell carcinoma		
with GOF mutation of SMO	PC increase transcriptional activator and stimulate proliferation	[63][64]
with GOF mutation of GLI2	PC increase transcriptional suppressor and inhibit proliferation	[63][64]

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