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Review

Freeing the brake: Proliferation needs primary cilium to disassemble

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Primary cilia are non-motile, microtubule-based, antennae-like organelle that protrude out from the cell surface and perform sensory function or transduce physiological signals in majority of the vertebrate cells. Cilia are assembled on basal bodies that are transformed centrioles. The assembly-disassembly of primary cilia may pose an additional measure on regulating cell cycle in vertebrate cells. While primary cilia are commonly found in differentiated or quiescent cells that are not cycling, disassembly of primary cilia may promote re-entry of these cells into the mitotic cycle, and support proliferation. Many cancer tissues or cancer-derived cells exhibit loss of primary cilia. However, primary cilia may also promote tumorigenesis in some contexts through growth-promoting signalling. This review will shed light on recent advancements of temporal coordination of ciliary disassembly and cell cycle progression, with a focus on how cilia loss may support tumorigenesis in various epithelial cancers.

Keywords. Cell cycle; hedgehog signalling; primary cilia disassembly; proliferation; tumorigenesis

1. Introduction

Eukaryotic cilia and flagella (these two terms can be used interchangeably) share similar basic structure as they both are microtubule-based, membrane-ensheathed tiny projections from the cell surface, and are assembled on basal bodies. Cilia are either motile or non-motile, which confer to motility or sensory functions. Motile cilia that are found in a multitude at specialized epithelial layers such as trachea, oviduct or ependyma regulate directional fluid flow, while sperm flagellum or flagella in lower eukaryotes provide swimming ability (Afzelius 2004). On the other hand, a non-motile primary cilium, present in the majority of vertebrate cells, functions as mechanosensors or chemosensors and transduces signalling pathways (Anvarian *et al.* 2019). A cilium consists of a microtubule (MT)-based axoneme assembled on a basal body that is a transformed centriole. A pair of centrioles, namely 'mother' and 'daughter', form the core of a centrosome, which is surrounded by γ-tubulin rich pericentriolar material (PCM) that emanates and organizes the cytoskeletal microtubular network in vertebrate cells. Generally, primary cilia (PC) are assembled in quiescent or interphase cells and must disassemble before those cells enter mitosis. Thus, the ciliogenesis program is regulated by both positive and negative regulators. While mechanistic details of various steps of cilia assembly were vividly investigated, the complexity of cilia disassembly remained understudied. Notably, several investigators have begun to unravel the temporal and spatial coordination of PC disassembly with the cell cycle in recent years and suggested that resorption of PC is necessary for a cell to proliferate [previously reviewed in Sánchez and Dynlacht (2016) and Goto *et al.* (2017)]. These and other recent studies prompted us to speculate that aberrant activation of PC disassembly in cells that are not meant to be proliferative, may now be cycling, which may subsequently support tumorigenesis at the early stage

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(schematically shown in figure 1). Here, we discuss the recent understanding of the molecular pathways to negatively regulating ciliogenesis with a focus on PC disassembly and the possible causal relationship of aberrant PC disassembly with tumorigenesis.

2. Function of primary cilium and ciliopathies

Primary cilia (PC) are commonly present in post-mitotic or quiescent cells and serve as a hub for sensory and signalling functions. In addition, PC are seen in proliferating cells (Han *et al.* 2008; Breunig *et al.* 2008; Higginbotham *et al.* 2013; Chang *et al.* 2019) and also in various cancer cells (table 1). PC transduce signals like that of hormones, growth factors, Ca^{2+} response or developmental morphogens from the extracellular environment to the interior of the cell, and are critical during development and in maintaining homeostasis of adult tissue. While Hedgehog (Hh) signalling is predominantly transduced via PC in vertebrates, PC may also coordinate other developmentally important signalling processes like Wingless (Wnt), G-protein-coupled receptors (GPCR), receptor-tyrosine kinases (RTKs), TGFβ, PDGF signalling, etc. (Anvarian *et al.* 2019). Consequently, defects in the structure of the cilia or in its function are associated with various disorders such as cystic kidneys, obesity, cardiac disorders, skeletal malformations, blindness, nervous system anomalies, etc. This collection of diverse and heterogeneous diseases caused due to genetic mutation(s) in one or more genes necessary for cilia assembly or function are collectively known as 'ciliopathies' (Brown and Witman 2014).

Sonic Hedgehog (SHH) signalling regulates embryonic development, cell fate, proliferation, tissue patterning, and homeostasis in mammals. Aberrant functioning of this pathway usually leads to congenital abnormalities. Its hyperactivation is proliferative and is common in many cancers (discussed in a later section). The Gli family of transcription factors, Gli1, Gli2 and Gli3 implement the transcriptional responses when SHH ligands bind to a transmembrane receptor Patched-1 (Ptch1), remove it from PC, and allow enrichment of Smoothened (Smo)-a GPCR family protein. In the absence of a ligand, proteolytically cleaved forms of Gli proteins are generated, particularly Gli3 that gets partially cleaved now gives rise to a repressor, repressing expression of SHH target genes. Also, another key regulator Suppressor of Fused (SUFU) sequesters Gli proteins from cytoplasm. Upon activation of SHH pathway, such processing of Gli proteins is inhibited. Also, SUFU-Gli complexes accumulate in PC and dissociate. Now, full-length Gli proteins, particularly Gli2 (with a minor role of Gli1) can function as transcriptional activators of the target genes (Gli1, Ptch1 are target genes). Both these processes are dependent on mature cilia, and shortening or loss of PC can abrogate transcriptional repression and activation in a tissue-specific manner (Bangs and Anderson 2017; Raleigh and Reiter 2019; Anvarian *et al.* 2019). Also, PC are critical to maintaining appropriate quiescence in adult stem cells such as myoblasts by restricting proliferation and by repressing G2 to M genes (Venugopal *et al.* 2020).

3. Structure of primary cilium and its assembly

The basal body of a primary cilium is attached to the apical membrane, and the axoneme, ensheathed within the ciliary membrane is protruded from the cell surface. Although the ciliary membrane is a continuation of the plasma membrane, it has its distinct composition of membrane components and receptors different from that of the plasma membrane (Larkins *et al.* 2011). Axonemes are made of nine doublet microtubules that are extended from the nine-fold symmetrical triplet microtubules of the centrioles. Among the two structurally distinguishable centrioles of a centrosome, the mother centriole that contains distal and sub-distal appendages is formed at least one cell cycle earlier than the daughter centriole (Azimzadeh and Marshall 2010). During cilia assembly, centrioles need to move toward the apical membrane, and the mother centriole needs to a) attach itself to the plasma membrane and b) accumulate membrane components via vesicle transport, to ultimately transform into the basal body. These two events may take place in two different ways as suggested by recent studies using advanced super-resolution microscopy. The 'extracellular pathway', which commonly takes place in polarized epithelial cells and the 'intracellular pathway', which is seen in fibroblasts (such as mouse NIH 3T3) or retinal pigment epithelial cells (such as hTERT-RPE1 or ARPE-19) (Lu *et al.* 2015; Garcia *et al.* 2018). The distal appendages of mother centriole turn into 'transition fibres' that help the attachment of mother centriole/basal body to the apical membrane. The connecting area between the distal end of the mother centriole and the base of axoneme is known as 'transition zone' that contains some very specific proteins (Reiter *et al.* 2012). Finally, the axoneme elongates utilizing the bi-directional cargo transport activity of intraflagellar transport (IFT)

machinery that is dependent on the anterograde and retrograde movement of Kinesin and Dynein motors respectively (Rosenbaum and Witman 2002; Ishikawa and Marshall 2017). Clearly, cilium assembly requires the participation of structural, enzymatic, microtubule-associated, membrane-binding, vesicle trafficking and motor proteins, in addition to membrane components and active cytoskeletal remodelling (Kim *et al.* 2010). The last two decades saw a revolution in the field with numerous studies to identify and characterize ciliary genes those when non-functional lead defect(s) in cilia assembly and maintenance, and interactions of various regulatory pathways of ciliogenesis. These studies are performed in various model systems starting from unicellular *Chlamydomonas* to mammalian cell culture and transgenic mice, and are aptly reviewed elsewhere (Seeley and Nachury 2010; Ishikawa and Marshall 2011; Reiter *et al.* 2012; Garcia *et al.* 2018; Wang and Dynlacht 2018).

4. Temporal coordination of ciliogenesis and cell cycle

Since most mammalian epithelial or endothelial cells and neurons do not undergo an active mitotic cycle, PC are permanently assembled in these cells. In the case of proliferating cells, PC are formed in quiescence (G0-phase) or in interphase (mostly during G1-phase), and are disassembled before G2/M transition. While the cell cycle is controlled by external factors such as growth-promoting factors, mitogens, etc., cell cycle checkpoints pose internal regulation on it. Complex molecular machineries make sure that growing cells satisfy the DNA damage checkpoint at G1/S transition, the G2/M checkpoint, and the spindle assembly checkpoint (SAC) to maintain genomic integrity. Interestingly, centrioles of a centrosome also duplicate during S-phase. Centriole duplication is initiated with each centriole assembling a daughter centriole at its proximal end, which matures during G2-phase. Finally, upon recruiting the PCM components, a cell builds two centrosomes at the onset of G2/M transition for assembling bipolar spindle. Strict regulations ensure precise duplication of centrioles only once, thereby preventing excess centriole generation and centrosome amplification, which otherwise may lead to attenuation of MT nucleation, loss of cell polarity and chromosome segregation error found in various cancers (Ganem *et al.* 2009; Nigg and Holland 2018).

Events like the assembly of PC during G0/G1-phase, mitogen-stimulated S-phase re-entry and subsequent PC disassembly, followed by centriole duplication and progression to mitosis are clear indications that there exists temporal coordination between ciliogenesis program and cell cycle (reviewed in Kobayashi and Dynlacht 2011; Izawa *et al.* 2015; Goto *et al.* 2017). These steps are mostly studied in cell culture of mouse NIH 3T3 fibroblasts and human hTERT-RPE1 (RPE1 as commonly abbreviated). Both are non-transformed and near-diploid cell types. Cellular quiescence or G0-phase can be readily established in these cells by removal of serum, the mitogen, from the growth medium for 24–48 h. During serum starvation, almost 85–90% of these cells assemble PC. Upon serum re-addition, majority of those cells disassemble (or resorb) PC, and re-enter cell cycle by crossing G1/S transition within 12–18 h. Therefore, serum starvation and subsequent addition became a robust assay for studying the function of the molecules that regulate PC disassembly. Notably, the asynchronous population of these cells growing in the presence of serum may have roughly 5–15% cells with PC. Even, 5–10% of cells that are in S-phase as judged by BrdU incorporation have PC (Majumder and Fisk 2013). Apparently, the majority of the cycling cells employ some molecular pathways to suppress cilia formation, which may or may not overlap with that of promoting PC disassembly, therefore suggesting two modes of negative regulation of ciliogenesis. This idea was discussed in some previous reviews with schematic diagrams of the molecules involved in cilia disassembly (Izawa *et al.* 2015; Sánchez and Dynlacht 2016; Goto *et al.* 2017). We aim here to discuss the recent advances in the area of negatively regulating ciliogenesis program, with a focus to determine if PC disassembly is a prerequisite for cell cycle progression and if aberrant loss of PC supports proliferation and tumorigenesis.

5. Suppression of PC assembly in cycling cells

Studies in the last few years showed various centriole-associated proteins, cell cycle regulators, proteasomal degradation pathways and cytoskeletal remodelling factors to suppress PC assembly in actively cycling cells. Usually, the growth medium for various cell types contains 5–15% serum, which is non-permissive for ciliogenesis. Inhibiting cell cycle regulators often pose ‘cell cycle arrest’ to restrict cells in G1-phase, thereby initiate a ciliogenesis program even in the presence of serum. Besides, there are cases when one or more ciliogenesis regulators affect cilia assembly or disassembly even in presence of serum independent of

the cell cycle. Sometimes, the later may halt cell cycle progression. However, it becomes tricky to determine if cell cycle arrest is causing aberrant cilia assembly or if the loss of suppression of PC to assemble delays cell cycle. Experimentally, rather than analyzing the affected asynchronous population one may need to examine cells that are in a certain cell cycle stage (such as in S- and/or G2-phase) without employing extrinsic synchronisation methods. Another strategy may be to examine if loss of function of the same protein affects serum-induced PC disassembly in RPE1 or NIH 3T3 cells. However, it is important to remember that these two events of negative regulation of ciliogenesis may not be affected similarly by the functional loss of a regulator.

During centriole assembly, the length of centrioles is largely controlled by centriolar proteins CPAP and CP110. CPAP helps in elongation of centriolar microtubules while CP110 acts as a 'cap' by localizing at the distal end of the centriole thereby inhibiting its growth after the desired length is achieved (Schmidt *et al.* 2009). During PC assembly, CP110 must be removed from the distal end of mother centriole (now basal body) to allow axoneme elongation, while its localization at daughter centriole remains unchanged (Spektor *et al.* 2007). Activities of two microtubule-associated proteins (MAP) modifying kinases TTBK2 and MARK4 are critical for CP110 removal from mother centriole after it is docked to the membrane by distal appendage proteins (Goetz *et al.* 2012; Tanos *et al.* 2013; Kuhns *et al.* 2013). CP110 along with its molecular interactors such as- CEP97, CEP290, Talpid3, etc suppress PC assembly in cycling cells (Spektor *et al.* 2007; Tsang *et al.* 2008; Kobayashi *et al.* 2014). Moreover, a kinesin 13 family protein Kif24 that interacts with CP110 at the mother centriole, also suppresses PC assembly likely via depolymerizing axonemal microtubules (Kobayashi *et al.* 2011). A recent investigation identifies that Kif24 recruits M-phase Phosphoprotein 9 (MPP9) to the distal end of centrioles where it binds to Cep97 of the CP110-Cep97 complex, and thus protects that complex from degradation. Moreover, TTBK2 that is recruited to the basal body during the early stage of ciliogenesis and may phosphorylate several distal appendage proteins (Bernatik *et al.* 2020), also targets MPP9 for phosphorylation, which triggers proteasome-mediated degradation of MPP9 and subsequent removal of CP110. Accordingly, loss of MPP9 leads to aberrant PC assembly in RPE1 cells growing in presence of serum and also in vivo, in proximal and distal tubules of mouse kidney cells resulting in developmental defects (Huang *et al.* 2018). Another in vivo study using CP110 knock out mice demonstrated that loss of CP110 affected the proper distribution of subdistal appendage proteins and docking of centriole to membrane leading to premature axoneme extension and ultimately loss of PC and impaired SHH signaling in the embryo (Yadav *et al.* 2016). Apart from this, a separate pathway of suppressing PC assembly in cycling cells is regulated by the mitotic kinase Nek2 that is activated during S/G2 transition. Interestingly, Nek2 phosphorylates Kif24 and activates it to suppress PC assembly (Kim *et al.* 2015b), indicating that Kif24 may link these two pathways in controlling cilia assembly during proliferation.

One of the major regulatory pathways to promote mitogen-stimulated PC disassembly is catalysed by HDAC6 that deacetylates axonemal microtubules, thereby destabilizing them leading to disassembly of PC (Pugacheva *et al.* 2007). HDAC6 is phosphorylated by Aurora Kinase A (AurkA), a centrosomal kinase that regulates centrosome maturation, mitotic entry, and spindle formation during G2/M-phases. AurkA gets activated via structural changes of the kinase domain leading to autophosphorylation of a conserved Thr 288 residue. Depletion of AurkA or Trichoplein, one of the activators of AurkA led to aberrant PC assembly in an asynchronously growing population of cells (Inoko *et al.* 2012). Such depletion also led to an arrest of those cells in a non-proliferating state, which could be reverted by ablating the ciliogenesis program. Moreover, the depletion of Trichoplein only showed little effects on cell cycle progression in HeLa cells that are unable to assemble PC (Inoko *et al.* 2012). Interestingly, the depletion of Ndel1, a dynein activity modulator, could phenocopy the effects of depleting the Trichoplein-AurkA module on PC assembly, as Ndel1 could work on stabilizing Trichoplein in a dynein-independent manner (Inaba *et al.* 2016). Independent studies showed that inactivation of Von Hippel-Lindau (VHL) tumor suppressor via mutation that is often seen in renal cell carcinomas induced HEF1/NEDD9 and AurkA activity through stabilizing hypoxia-inducible factors 1 and 2 (HIF1 and HIF2). Such inactivation of VHL was considered the reason for axonemal microtubule destabilization and regression of PC in those cancer-derived cells (Xu *et al.* 2010). Similar to epithelial cells, HIF1α also activates AurkA in endothelial cells promoting PC resorption upon modulation of shear stress (Ki *et al.* 2020), which is discussed in the following section.

Studies from our group suggested novel regulatory pathways of suppressing ciliogenesis program in

cycling cells by Voltage-Dependent Anion Channel (VDAC) proteins that are mitochondrial porins and best known to control mitochondrial bioenergetics. Depletion of VDAC1 and VDAC3, but not VDAC2, in RPE1 cells led to aberrant PC assembly in roughly 70% cells of asynchronous population, compared to 15–20% of control cells treated similarly. There was significant increase in PC-containing RPE1 cells treated with either VDAC1 or VDAC3-specific siRNAs compared to the control transfection, which were in S-phase as judged by BrdU incorporation (Majumder and Fisk 2013; Majumder *et al.* 2015). These observations suggest that VDAC1 or VDAC3 facilitate activation of one or more negative regulators of ciliogenesis to suppress PC assembly. Though the mechanistic detail of VDAC-mediated suppression of PC assembly is not clear yet, it is hypothesized that either the centrosomal pool of VDAC1 or VDAC3 (Majumder *et al.* 2012, 2015), or VDAC molecules localized at the mitochondrial outer membrane perhaps serve as a molecular scaffold to help in such activation. Another testable model to explain these observations may be altered cellular metabolic status due to the depletion of VDACs leading to aberrant ciliation in non-permissive conditions. Indeed, a separate study showed that mitochondrial stress due to mitochondrial fission or ROS generation subsequently activated AMPK and autophagy, thereby induced aberrant PC assembly (Bae *et al.* 2019). Similarly, impairment in mitochondrial function either due to the genetic aberration of mitochondrial gene(s) or upon treating cells with Rotenone, an inhibitor of complex I of the mitochondrial respiratory chain led to significant increase in the length of motile or non-motile cilia likely due to altered cellular metabolic status (Burkhalter *et al.* 2019). Furthermore, the reduction in SIRT2 function led to aberrant PC assembly in RPE1 cells in presence of serum which, in turn, arrested cells in G0/G1-phase likely via inactivation of the mTOR signaling pathway (Lim *et al.* 2020). Also, treating highly proliferative tumorigenic kidney epithelial cells with Rapamycin restores PC assembly and length, and subsequently inhibits cell proliferation (Jamal *et al.* 2020). Although several elegant studies were conducted to comprehend the relationship between ciliogenesis and mTOR signaling pathway or autophagy [reviewed in (Wang *et al.* 2015; Pampliega and Cuervo 2016; Lai and Jiang 2020)], future investigations may warrant to unravel the translational potential of this complex regulation of cilia biology.

Autophagy and ubiquitin-proteasome system are the two major protein degradation systems in eukaryotes, and like autophagy, the proteasome pathway that controls both protein degradation and protein processing also takes a critical role in regulating both assembly and disassembly of PC (Izawa *et al.* 2015). The ubiquitin-proteasome modulators, ubiquitin-conjugating enzymes, substrate recognizing molecules and deubiquitinases were found to localize at centrosomes or at PC to pose a spatial control on protein degradation at these specific locations independent of overall cellular control (Gerhardt *et al.* 2015; Izawa *et al.* 2015; Kim *et al.* 2016). Such cilia/centrosome specific proteasomal activities usually suppress the PC assembly in cycling cells via regulating multiple proteins. For example, proteasome-mediated degradation regulates Cep97 and Trichoplein, where CUL3-RBX1-KCTD10 and CRL3-KCTD17 function as E3 ligase respectively, to suppress ciliogenesis program in cycling cells (Kasahara *et al.* 2014; Nagai *et al.* 2018). On the other hand, the Trichoplein-AurkA pathway is stabilized by deubiquitinase USP8 upon phosphorylation by epidermal growth factor receptor (EGFR) kinase to suppress PC assembly in growing cells (Kasahara *et al.* 2018). The anaphase-promoting complex (APC) that functions with two coactivators Cdc20 and Cdh1 (APC^{Cdc20} and APC^{Cdh1}) in a temporally coordinated manner serves as the key ubiquitin E3 ligase during mitosis. Notably, both these complexes were found to localize near centrosomes/basal bodies in interphase cells and to regulate ciliogenesis program (Wang *et al.* 2014; Gupta *et al.* 2017), although the temporal coordination among these two complexes at centrosomes/basal bodies requires further investigation.

Thus, the majority of these studies discussed here demonstrated that aberrant PC assembly in cycling cells affected cell cycle, and functional interactions between several molecular regulators of ciliogenesis are the key to keep cells dividing and proliferating. However, loss of PC abrogates SHH signaling in postnatal hippocampal progenitors and induces cell cycle exit in astrocyte-like neural precursors (Han *et al.* 2008; Breunig *et al.* 2008). Another recent study shows that cerebellar granule neuron progenitors (GNPs) proliferate in response to SHH signaling that is transduced via PC (Chang *et al.* 2019). Thus, PC transduces SHH signaling in the postnatal neuronal epithelial cells to keep these cells proliferating, and only a transient PC resorption at G2-M transition may likely ensure the mitotic events to take place followed by cell division. The requirement of PC in postnatal cerebellum development is also reflected in the case of SHH-induced medulloblastoma, a pediatric brain tumor arising from

the cerebellum, which relies on the presence of PC (Han *et al.* 2009).

Overall, suppression of the ciliogenesis program in proliferating cells may be initiated in S-phase or G2-phase but must continue until the end of mitosis. In tissues where Hh signaling controls cellular proliferation then it is important to keep the dynamics of assembly and disassembly in a timely manner. Nevertheless, the molecular mechanism involved in the suppression of PC assembly is likely to be cell cycle-regulated, and important therapeutic target to inhibit cell proliferation.

6. Apical actin network negatively regulates ciliogenesis

In 2010, a comprehensive genome-wide siRNA screen to identify regulators of ciliogenesis in human RPE1 cells revealed that depletion of several actin cytoskeletal remodelling factors and vesicle trafficking regulators may affect ciliogenesis both positively and negatively (Kim *et al.* 2010). Among them, functional assays validated that actin filament severing molecules (such as Gelsolin) were required for cilia assembly, whereas Actr3 that helps Arp2/3-nucleated branched actin network or PLA2G3 that is implicated in endocytic recycling pathway negatively regulated ciliogenesis and PC length. Thus, it was conceived that disrupting apical branched actin network promoted PC assembly by allowing centriole movement towards the ciliary site and stabilizing the pericentrosomal preciliary compartment (PPC), a reservoir for transmembrane proteins and membrane components required for ciliogenesis (Kim *et al.* 2010). Accordingly, treating cells with actin cytoskeleton depolymerizing agents such as cytochalasin D or cytochalasin B leads to aberrant assembly of longer PC in cycling cells, independent of serum (Kim *et al.* 2010; Drummond *et al.* 2018). Further investigations supported this notion demonstrating that loss of function of the branched actin nucleator Arp3 and N-WASp or small GTPase Cdc42 that promotes Arp2/3- and WASp-mediated actin filaments nucleation led to increase in PC length and altered Hh signaling in primary mouse dermal cells or NIH 3T3 cells (Drummond *et al.* 2018). Consistent with these ideas, Cao et al showed that miR-129–3p, a microRNA conserved in vertebrates, downregulated CP110 and repressed branched F-actin formation, and thereby promoted PC assembly in cycling cells and subsequent cellular proliferation (Cao *et al.* 2012). An apparently surprising observation was the increase in ciliated RPE1 cells in presence of serum upon treatment with Jasplakinolide that promotes actin polymerization. Interestingly, it was observed that Jasplakinolide-induced actin remodelling forced cells to attain quiescence due to the inactivation of the transcriptional co-activator YAP that promotes cell proliferation (Nagai and Mizuno 2017). Indeed, another study demonstrated that kinases LIMK2 and TESK1 phosphorylate actin filament severing protein Cofilin, which results in its degradation leading to loss of actin remodelling that helped vesicle trafficking towards the basal body (Kim *et al.* 2015a). Moreover, cytoskeletal remodelling that led to change in cell shape inactivated YAP (or its paralogue TAZ) pathway leading to reduced expression of AurkA and Plk1 and aberrant PC assembly establishing a negative role of YAP/TAZ in ciliogenesis (Kim *et al.* 2015a). Notably, the Hippo tumour-suppressor pathway is the key negative regulator of YAP/TAZ activity through phosphorylation-dependent cytoplasmic retention and destabilization. Thus, lack of such a tumor suppressor pathway may result in loss of PC assembly via transcriptional regulation aided by actin cytoskeletal remodelling. A recent study showed that depletion of leucine-zipper actin-stabilizing protein LUZP1 that also localizes to centrosomes/basal bodies in addition to F-actin increases the levels of MyosinVa and destabilizes actin filaments. Both these pathways promote transport of preciliary vesicle to mother centriole and thereby aberrant PC assembly in non-permissive conditions and elongation of PC (Gonçalves *et al.* 2020). LUZP1 interacts with truncated SALL1 a transcription factor that is often mutated in Townes-Brocks Syndrome (TBS), and this truncated SALL1 expedites proteasome mediated degradation of LUZP1 (Bozal-Basterra *et al.* 2020). These two studies reinstated how in cycling cells PC assembly is suppressed by actin cytoskeletal dynamics and its modulators. Another important aspect of recent studies is how actin filament polymerization may facilitate active removal of PC, which is discussed in the following section. Thus, actin cytoskeleton regulators appear to be attractive therapeutic targets in treating pathologies associated to ciliary dysfunction.

7. Regulation of serum-induced PC disassembly

Few studies have suggested that serum-stimulated PC disassembly may occur in two waves following serum addition to the serum-starved cells. The first wave of PC disassembly takes place within 2 h of serum

stimulation, which likely induces G0 to G1 transition and is needed for subsequent entry to S-phase for both DNA synthesis and centriole duplication to occur (Pugacheva *et al.* 2007; Plotnikova *et al.* 2011; Wang *et al.* 2013). On the other hand, 'second wave of cilia disassembly' that happens less synchronously after 12–24 h of serum addition ensures cilia removal before completion of G2-phase, and is needed for those cells to enter mitosis (Kim *et al.* 2015b).

The most studied PC disassembly factor is the AurkA-HDAC6 module that is regulated at various levels by other molecular factors. AurkA is a functional homolog of *Chlamydomonas reinhardtii* protein kinase CALK that played a significant role in flagellar disassembly (Pan *et al.* 2004). In vertebrates, apart from its mitotic roles, AurkA phosphorylates HDAC6 upon activation by HEF1/NEDD9 after serum re-addition (Pugacheva *et al.* 2007). Subsequent studies showed that Ca^{2+}/Calmodulin (Cam) and Pifo can also activate AurkA and thereby HDAC6, during PC resorption (Plotnikova *et al.* 2010, 2012; Kinzel *et al.* 2010). HDAC6 can also be activated by Polo-like kinase 1 (Plk1), another mitotic kinase that perform non-mitotic function to promote PC disassembly (Seeger-Nukpezah *et al.* 2012; Wang *et al.* 2013). Also, Plk1 interacts with Dishevelled 2 (Dvl2) to initiate serum-induced PC disassembly through stabilizing HEF1 following non-canonical Wnt5a ligand stimulation (Lee *et al.* 2012). In a separate pathway, Plk1-mediated phosphorylation activated KIF2A, a member of mammalian kinesin 13 family, to perform axonemal microtubule depolymerization and destabilization that facilitated PC disassembly (Miyamoto *et al.* 2015). The recruitment of KIF2A is regulated by mother centriolar protein Cep170 and the primary microcephaly associated protein WDR62. Thus, loss of function of WDR62 prevents KIF2A recruitment to the basal body and results in retarded PC disassembly and longer PC leading to delayed cell cycle progression, reduced proliferation and premature differentiation of neural progenitor cells (Zhang *et al.* 2019). Like suppressing PC assembly in growing cells, Nek2 mediated phosphorylation of Kif24 also promoted PC disassembly likely via microtubule depolymerization, independent of AurkA-HDAC6 module (Kim *et al.* 2015b). Thus, HDAC6, KIF2A and KIF24 are all attributed to their ability to promote axonemal MT depolymerization although future investigations are needed to understand the temporal interaction between the activities or mutual dependence of these three key proteins. To ensure complete resorption of PC, Nek2 activity removes distal appendage proteins from the mother centrioles before entering to mitosis. Consequently, loss of Nek2 activity in RPE1 cells resulted in improper removal of distal appendage proteins such as Cep164, Cep123, and LRRC45, which remained at mother centriole as a remnant of cilia, and promoted cilium reassembly readily after cell division due to the asymmetric inheritance of cilia assembly components (Paridaen *et al.* 2013; Viol *et al.* 2020).

Functioning as a cellular antenna, PC on endothelial cells sense and transduce shear stress due to blood flow. An interesting recent study suggested that a complex functional interaction between Joubert syndrome associated centriolar protein Cep41, tubulin polyglutamylase TTLL6, and cytoplasmic carboxypeptidase 5 (CCP5) that may function as a deglutamylase regulate axonemal microtubule glutamylation in endothelial cilia examined in human umbilical vein endothelial cells (HUVEC). Here, shear stress-induced Cep41-mediated dynamic glutamylation activated AurkA via HIF1α promoted resorption of endothelial primary cilia, which subsequently induced VEGF pathway and shear stress-induced angiogenesis (Ki *et al.* 2020). However, future investigations are required to delineate how opposing activities of Cep41 and CCP5 may modulate MT glutamylation, how axoneme glutamylation, unlike acetylation, helps in promoting ciliary disassembly, and how the process of PC disassembly drives angiogenesis.

Inhibiting PC disassembly promoting proteins usually delay cell cycle re-entry in a cilium-dependent manner. Notably, depletion of Mps1 kinase, an essential SAC component and also an important centriole assembly factor (Sawant *et al.* 2015), almost completely inhibited serum-induced PC disassembly and entry of those cells to S-phase (Majumder and Fisk 2013). This function of Mps1 is independent of its role as a spindle assembly checkpoint as the cells were taken to quiescence within 12 hrs of siRNA treatment. Depletion of VDAC3, one of the three mitochondrial VDACs, also markedly inhibited PC disassembly upon serum stimulation, possibly via controlling the centrosomal function of Mps1 (Majumder *et al.* 2012; Majumder and Fisk 2013). Depletion of another cell cycle kinase CCRK led to the accumulation of its substrate Intestinal cell kinase (ICK) at ciliary tips, altered ciliary transport, inhibited PC disassembly, and subsequently delayed cell cycle re-entry in mouse NIH 3T3 fibroblasts (Yang *et al.* 2013). Notably, cell cycle kinases such as AurkA, Mps1, Nek2 or CCRK are often overexpressed in human cancers, and several studies indicated that targeting these kinases by genetic means or by small molecule inhibitors inhibited

tumorigenesis. While VDAC3 was not always examined in cancers, VDAC1 is known to be overexpressed in many cancers. An interesting recent study on patient samples of clear cell renal cell carcinoma (ccRCC) and RCC derived cells indicated that presence of a HIF1α induced c-terminally cleaved form of VDAC1 (VDAC1-ΔC; lacked c-terminal 69 residues), and not the full-length VDAC1, was associated with the loss in PC. Another cohort where PC was either present or re-expressed upon treatment lacked VDAC1-ΔC (Fabbri *et al.* 2020). This study indicates that while the hypoxia-induced form of VDAC1 does not affect glycolysis, it likely forces PC resorption via a yet unknown molecular pathway in renal cell carcinoma. Overall, it is tempting to speculate that targeting these molecules during the early stage of tumorigenesis may help non-proliferating cells to retain their PC, which may be an additional therapeutic strategy against tumorigenesis.

The dynamicity of PC length is maintained by the continuous turnover of major ciliary components aided by balancing the activity of anterograde and retrograde motor-dependent IFT function. Nde1 that interacts with LC8, a dynein light chain component, regulates the shortening of PC. Thus, the depletion of Nde1 not only led to a dramatic increase in PC length but also inhibited serum-induced cell cycle re-entry of NIH 3T3 cells. The later was rescued when cells were co-depleted for IFT88 or IFT20, which are essential for PC assembly (Kim *et al.* 2011b). It was later discovered that Cdk5 primed Nde1 for proteasome-mediated degradation via the E3 ligase activity of FBXW7 during serum-induced G0 to G1 transition of RPE1 cells (Maskey *et al.* 2015). Importantly, Doobin et al demonstrated that shRNA-mediated depletion of Nde1 in rat brain inhibited proliferating neural progenitor cells to progress through both G1-S and G2-M transitions likely due to the presence of PC. There exists a demarcation of the function of Nde1 and Ndel1, the two paralogues, in negatively regulating ciliogenesis and neurogenesis. While overexpression of Ndel1 compensated the role of Nde1 in PC resorption and G1-S transition, it failed to do the other role of Nde1 in completing interkinetic nuclear migration in radial glial progenitor cells to avoid a block in G2-M transition and proliferation (Doobin *et al.* 2016). Another dynein light chain subunit Tctex1 was known to perform dynein independent cytoplasmic function when phosphorylated at Thr 94 possibly by protein kinase C (Chuang *et al.* 2005). Upon serum stimulation, phosphorylated Tctex1 was recruited to the ciliary transition zone and promoted PC disassembly. A dominant non-phosphorylable mutant of Tctex1 (Tctex1-Thr94Ala) not only inhibited PC resorption, but delayed the re-entry of those cells to S-phase as judged by BrdU incorporation, and also the subsequent cellular proliferation (Li *et al.* 2011).

Oral-facial-digital syndrome 1 (Ofd1) usually localizes at centriolar satellites and needs to be degraded by autophagy to facilitate PC assembly (Tang *et al.* 2013). Interestingly, a novel function of CPAP was identified in promoting PC disassembly, via serving as a scaffold for various PC disassembly promoting factors such as AurkA, Nde1 and Ofd1 at the ciliary base. A mutation in CPAP that is seen in Seckel syndrome with microcephaly, resulted in longer PC, retarded PC disassembly, delayed cell cycle re-entry and subsequently reduced proliferation in neural progenitor cells (NPCs) during development, which ultimately led to premature differentiation (Gabriel *et al.* 2016). Also, cAMP-dependent protein kinase A (PKA) that was shown to localize at PC, which phosphorylated Nek10 to target for proteasome-mediated degradation guided by E3 ubiquitin ligase CHIP, and thereby stimulate PC resorption. Increased expression of CHIP is associated with malignant tumors and may be correlated with reduced Nek10 level and aberrant loss of PC in those cancer cells (Porpora *et al.* 2018).

Consistent with the idea that reduced F-actin may support PC assembly in RPE1 cells, we observed marked reduction in actin filaments (as judged by phalloidin staining) in RPE1 cells that were serum-starved for 24 h compared to that were growing in presence of serum (data not shown). Expectedly, a brief treatment of such serum-starved cells with cytochalasin D before serum stimulation delayed PC disassembly, likely by maintaining vesicular trafficking towards the basal body (Li *et al.* 2011). Another study showed that phospho-Tctex1 helped serum-stimulated activation of Cdc42 GTPase, which regulate Arp2/3 and Anxa2 protein complexes responsible for branched actin cytoskeletal remodelling at the ciliary base. Such cytoskeletal remodelling promoted endocytosis of membrane components from the ciliary pocket in a Clathrin-, Dynamin-, and Rab5-dependent pathway, and therefore facilitated ciliary resorption upon serum addition (Saito *et al.* 2017).

In addition to destabilization of the axonemal microtubules, disassembly of PC also requires shortening of the ciliary membrane through either endocytosis or degradation or shedding. Two recent studies indeed demonstrated the removal of ciliary vesicles that get pinched off from the distal tip of PC upon serum stimulation (Nager *et al.* 2017; Phua *et al.* 2017). The

ciliary decapitation is mediated by intra-ciliary actin polymerization at a site determined by the distribution of phosphatidylinositol 4,5-bisphosphate [PI(4,5)P2], and aided by actin cytoskeletal modulating factors (Phua *et al.* 2017). Small GTPase Rab7 helps in intraciliary actin polymerization upon serum stimulation to facilitate deciliation in RPE1 cells, although such activity of Rab7 is independent of its established role in endo-lysosomal maturation (Wang *et al.* 2019). Consistently, a live cell study demonstrated rapid deciliation i.e. shedding off the whole cilia containing axoneme and ciliary membrane in the growth medium is the major mode of serum-induced cilia loss in IMCD3 mouse polarized epithelial cells (Mirvis *et al.* 2019). Clearly, these studies have opened a novel and exciting avenue of ciliary disassembly that relies on actin filament polymerization inside PC that was thought to be devoid of F-actin. In fact, actin reorganization inside PC regulated by both MT and actin-binding formin FHDC1 was found to control PC length (Copeland *et al.* 2018). However, future investigations in this area may need to answer some important questions such as (a) how deciliation and PC resorption are temporally coordinated during cell cycle re-entry, (b) if both these events take place in different cell types, and (c) if deciliation that is faster than the other, is favoured in certain cells such as in postnatal neural progenitor cells where a possibly transient PC removal prior to mitosis or a highly dynamic PC assembly and disassembly is necessary to maintain proliferation (Lepanto *et al.* 2016).

From these discussions and from several other studies which could not be discussed due to space constraints we realized that any treatment that inhibited PC disassembly or promoted longer cilia such as- brief treatment of serum-starved cells with cytochalasin D right before serum re-addition, ectopic expression of a constitutively active form of small GTPase Rab8a that leads to longer cilia, depleting the activity of Nde1 or Tctex1, etc, ultimately stalled mitogen-induced cell cycle re-entry or G0/G1 to S-phase transition (Kim *et al.* 2011b; Li *et al.* 2011). Such delay in cell cycle progression could often be reversed by ablating primary cilia. Likewise, depletion of CEP164, a distal appendage protein essential for PC assembly, in quiescent cells markedly increased the percentage of S-phase cells after being serum-stimulated (Slaats *et al.* 2014). Also, forced PC resorption in neuronal cells following injury by hypoxia or optic nerve transection led to aberrant cell cycle re-entry and proliferation of those cells, which ultimately underwent apoptosis. Inhibiting PC disassembly factors restored PC, helped in neuronal survival and decreased the phosphorylation of retinoblastoma (Rb), a master switch for cell cycle re-entry (Choi *et al.* 2019).

Thus, our discussion indicated that cell cycle progression depends on PC disassembly, and aberrant PC disassembly may promote the proliferation of cells that are not supposed to cycle in healthy tissues otherwise (Figure 1). Such proliferation may lead to a pathological situation such as epithelial-to-mesenchymal transition (EMT) associated with renal fibrosis (Slaats *et al.* 2014) or neuronal apoptosis that facilitates neurodegeneration (Choi *et al.* 2019). Overall, the majority of the studies point to the fact that aberrant activation of PC assembly in growing cells almost always inhibits cell proliferation.

8. Primary cilia and tumorigenesis

The discussion until now suggested that cells achieve growth advantage when PC are lost. Therefore, highly proliferative cancer cells generally need to lose their PC. Similarly, cells that are in a non-proliferative and non-mitotic state, need to disassemble PC for proliferation. Thus, forced PC loss could be one of the driving reasons during the early stage of tumorigenesis. Indeed, several studies showed the loss of PC in neoplastic tissues and cancer-derived cell lines, compared to healthy tissues or control cells (Table-1). Yet, it remained debatable to suggest a causative role of PC loss in promoting tumorigenesis because of two major reasons. Firstly, SHH signalling promotes proliferation in some adult tissues, and is oncogenic in several cancers (Raleigh and Reiter 2019). Secondly, since tumor cells usually proliferate fast, and do not spend enough time in interphase to allow assembly, fewer PC in tumor cells could just be a cell cycle phenomenon.

An elegant review by Raleigh and Reiter described how Hh signalling (or SHH signalling) may promote tumorigenesis (Raleigh and Reiter 2019), a topic which we will briefly talk here. As discussed in an earlier section, the canonical SHH signalling is mediated by Smo enrichment at PC and subsequent processing of Gli proteins (particularly Gli2 and Gli3) inside cilia. However, Hh signalling can also occur via aberrant or non-canonical pathways, which are cilia-independent, and involve overexpression of the ligand, or genetic mutation in SHH-pathway regulators Patch1, Smo or SuFu (Raleigh and Reiter 2019). If PC has any role in modulating such non-canonical Hh pathways or whether such pathways act in parallel to canonical Hh signalling during development or to support

proliferation or tissue homeostasis via PC are not known yet. Also, Gli transcriptional factors are involved in cancer progression through cross-talk with other Smo-independent pathways such as TGFα pathway, in addition to the canonical SHH pathway (Hassounah *et al.* 2017). Thus, presence or absence of PC may process the signalling in two different ways based on any oncogenic predisposition or additional (or secondary) genetic alteration or differential expression of Hh-regulatory genes, which may ultimately result deregulated activation or loss of repression of Hh-target genes (Hassounah *et al.* 2012). Indeed, two exciting studies demonstrated that PC-mediated SHH signalling may both mediate and suppress SHH-signalling dependent tumorigenesis in mouse models of basal cell carcinoma (BCC) and medulloblastoma (Han *et al.* 2009; Wong *et al.* 2009). In these cases, when PC were ablated in cells that are induced by activated Gli2, then Hh-pathway remains 'on' without ligand, while activated Smo drives tumorigenesis-promoting Hh-pathway only in presence of PC. Inpp5e, an inositol polyphosphate 5-phosphatase that hydrolyses PtdIns(4,5)P2 and PI 3-kinase product PtdIns(3,4,5)P3 and helps in PC maintenance. Conditional deletion of Inpp5e in a murine model of constitutively active Smo-driven medulloblastoma promoted PC loss, suppressed cell proliferation, slowed tumor progression, and reduced SHH signalling (Conduit *et al.* 2017). Since Hh pathway is commonly misactivated in various cancers, Hh pathway antagonists, Gli inhibitors and Smo inhibitors (Vismodegib and Sonidegib, two most potent Smo-inhibitors) are often tested as anti-cancer therapies (Hassounah *et al.* 2012; Raleigh and Reiter 2019). Now, these inhibitors will work only when SHH pathway normally operates in those cancer patients. Consistent with this idea, defects in PC assembly or loss of PC posed resistance to Smo inhibitors in medulloblastoma (Zhao *et al.* 2017). Similarly, disrupting INTU, a planner cell polarity effector, in an oncogenic Smo-driven BCC murine model prevented the formation of BCC through suppressing PC formation and Hh signalling (Yang *et al.* 2017). Thus, in cells where SHH signaling drives proliferation (such as in polarized cells), Hh pathway activating mutations may promote tumorigenesis that requires PC (Raleigh and Reiter 2019).

On the other hand, table 1 summarizes those studies that demonstrated loss or reduction of PC in tissues or cells of various epithelial cancers, or restoring PC by genetic means inhibited tumorigenic progression *in vitro* and in xenograft model. Such negative correlation of the presence of PC and tumorigenesis is commonly seen in melanoma, Glioblastoma, PDAC, Breast tumorigenesis, prostate and thyroid cancers (Table 1). The absence of PC may be caused due to (a) defect in PC assembly or maintenance, (b) hyperactivity of PC disassembly promoting factors or (c) lack of quiescence phase in hyperproliferative cancer cells. It can be said that either non-canonical Hh signalling operates in these tissues, which becomes aberrantly hyperactive or PC loss causes insensitivity to environmental repressive signals, ultimately causing or facilitating proliferation during tumorigenic progression. SHH signaling is dispensable or in several cases may be inhibitory for the majority of these tumors (Raleigh and Reiter 2019). Indeed, ablation of PC in a polyoma middle T mouse model of breast cancer led to earlier tumor formation and a more aggressive type of tumor that grew faster with a higher metastatic ability (Hassounah *et al.* 2017). Ablation of PC also led to aberrant activation of Hh target genes likely because those cells could not process the Gli transcription factors resulting in the repressor form (Hassounah *et al.* 2017). Thus, it is not only a cell cycle event, instead of a defect in the ciliogenesis program of PC and PC-mediated signalling, which are deregulated in cancers upon loss of PC due to aberrant disassembly. Also, the aberration of other signalling pathways (such as- Wnt, TGFβ, PDGFα etc) due to loss of PC may also contribute to tumorigenesis. Indeed, an elegant study using a mouse melanoma model showed that hyperactivation of histone methyltransferase EZH2, frequently overexpressed in human melanoma, led to metastatic melanoma by promoting tumorigenic Wnt/β-catenin signaling pathway (Zingg *et al.* 2018). In conjunction with oncogenic drivers such as BrafV600E or NRasQ61K, a gain-of-function mutant of EZH2 ($EZH2^{Y646N}$) drove silencing of several genes critical for PC assembly, and thereby significant loss of PC in benign melanocytic lesions. Conversely, pharmacological blocking of the catalytic activity of EZH2 restores PC and inhibited growth in malignant melanoma (Zingg *et al.* 2018).

The correlation between fewer PC in more aggressive tumors (Ciliation Index) was suggested to be utilized as a diagnostic marker/tool in addition to other histological assays (Table-1). Moreover, it will be interesting to examine if restoring PC assembly in those cancers can be a therapeutic strategy, at least to inhibit proliferation and tumor growth. In fact, several cancer cell lines (pancreas, lung, kidney, breast) were treated with a panel of small molecule inhibitors and repurposing drugs, and several of these compounds reduced PC number and tumor

Table 1. Studies that showed negative correlation between Primary cilia and cancer

Type of cancer	Brief observation and outcome of the study	References
Melanona: malignant cancers of melanocytes which spreads to other body parts	Presence of PC was evaluated in 68 melanocytic lesions ranging from Spitz nevi to atypical Spitz tumor and Spitz melanoma, which share similar histology. PC were significantly decreased in melanoma compared to other samples. Ciliation index (CI; % PC per melanocyte) was suggested as a diagnostic tool in melanoma	Lang *et al.* (2020)
	Presence of PC was evaluated in biopsy from 6 malignant acral melanoma and 7 acral nevi were examined and a significant decrease in CI in the first type was observed	Love *et al.* (2019)
	In murine melanoma models, gain-in function of EZH2, a transcriptional regulator commonly overexpressed in melanoma, led to malignancy from benign BrafV600E- or NrasQ61K- expressing melanocytes, likely by silencing of PC integrity genes and subsequent loss of PC. Conversely, blocking EZH2 activity restored PC, and inhibited growth inhibition in malignant melanoma	Zingg *et al.* (2018)
	Presence of PC was determined in 62 samples that included cutaneous melanocytic nevi, melanoma in situ, invasive melanoma, and metastatic melanoma. PC were found in nearly all melanocytes of 22 melanocytic nevi samples, but were mostly lost in 16 cases of melanoma in situ,16 primary invasive melanomas, and 8 metastatic tumors, each associated with a cutaneous primary lesion	Kim *et al.* (2011a)
Glioblastoma multiforme (GBM): aggressive cancer of astrocytes	Few GBM cell lines that commonly overexpress HDAC6, which is corelated with reduced PC in GBM, were treated with HDAC6 inhibitor Tubastatin A. Such treatment downregulated SHH signalling, clonogenic and migration properties of these cells, accelerated temozolomide-induced apoptosis, and decreased expression of mesenchymal markers in GBM cells	Urdiciain *et al.* (2019)
	Ablation of PC in primary human astrocytes led to redistribution of lysophosphatidic acid receptor 1 (LPAR1) that are commonly accumulated to PC on plasma membrane, and thereby association of LPAR1 with Gα12 and Gαq, which drove tumorigenesis. Inhibition of LPA signalling in deciliated astrocytes or GBM cells reduced tumorigenesis both in vitro and in xenograft model. Such inhibition reduced tumor growth in an intracranial GBM model	Loskutov *et al.* (2018)
	7 surgically resected human GBM tissue samples were examined for PC structure using IIF and EM, which showed structural aberration in PC	Moser *et al.* (2014)
	Depletion of CCRK that is overexpressed in GBM-derived cells restored PC and inhibited cell proliferation	Yang *et al.* (2013)
	5 normal human astrocytes and 5 GBM-derived cell lines were examined for presence of PC and their structure using IIF and EM, which showed all GBM cells either have fewer PC or had structural aberration in PC	Moser *et al.* (2009)

Table 1 (continued)

Type of cancer	Brief observation and outcome of the study	References
Pancreatic Ductal Adenocarcinoma (PDAC)	Ablation of PC from insulin-producing β cells of pancreas by removing Ift88 led to spontaneous auto-activation of EphA3, defective insulin secretion, polarity defects and epithelial-to-mesenchymal transition	Volta *et al.* (2019)
	Depletion of HDAC2 in PDAC-derived cells decreased AurkA expression and thereby restored PC	Kobayashi *et al.* (2017)
	PC frequency was increased in adenocarcinoma of lung, colon, pancreas, and in follicular lymphoma, with structural abnormalities in PC (axoneme length & branching, multiple basal bodies) compared to normal tissues	Yasar *et al.* (2017)
	PC were analyzed in tissues from normal pancreas, chronic pancreatitis, intraductal papillary-mucinous neoplasia, and PDAC, and in primary human pancreatic stellate cells (PSC) and pancreatic cancer cell lines by IIF. Gradual decrease in PC from normal to neoplastic epithelial cells, with increase of PC in stroma were observed. HH receptors were redistributed in those PC	Schimmack *et al.* (2016)
	Sustaining increased HH activity by overexpressing Gli2 in β cells of pancreas in a mouse model showed impaired insulin secretion, and development of undifferentiated pancreatic tumors	Landsman *et al.* (2011)
	PC were mostly absent in pancreatic cancer cells and pancreatic intraepithelial neoplasia (PanIN) lesions from PDAC, even in non-proliferating cells compared to normal duct, islet, and centroacinar cells, from mouse models of PDAC driven by oncogenic Kras allele	Seeley *et al.* (2009)
Breast cancer	Inhibiting PC led to earlier tumor formation, faster tumor growth rate, higher grade tumor formation, and increased metastasis in a polyoma middle T (PyMT) mouse model. Expression of Hh-target genes was increased through loss of Gli repressor and activation of Hh signalling via cross-talk with TGF-alpha	Hassounah *et al.* (2017)
	PC were examined in cancer cells as well as their surrounding stromal cells from 86 breast cancer patients, and compared with normal. PC were reduced in both premalignant lesions as well as in invasive cancers, and also in stroma, but this did not increase proliferative index	Menzl *et al.* (2014)
	PC were not observed in epithelial cells of breast cancer tissues, and also not in cancer cells in the orthotopic and metastatic xenograft tumors of breast cancer, though these cells are non-proliferative (Ki-67 negative)	Nobutani *et al.* (2014)

Table 1 (continued)

Type of cancer	Brief observation and outcome of the study	References
Prostate cancer	PC were examined in human prostate tissues from normal prostate, and various stages of prostate cancer including prostatic intraepithelial neoplasia (PIN), and invasive prostate cancer, which showed reduced PC number and length in PIN and invasive cancer compared to normal	Hassounah *et al.* (2013)
	TACC3 level that is often elevated in prostate cancers and this elevated level inhibits PC assembly. TACC3 depletion reduced tumorigenesis	Qie *et al.* (2020)
Hepatocellular Carcinoma (HCC)	Inhibition of PC assembly by Ift88 silencing showed protumor effects- it enhanced proliferation, migration, and invasion ability of HCC-derived cells; accelerated tumor growth and increase of autophagic flux in xenograft model of HCC	Liu *et al.* (2019)
Thyroid cancer	Mice with thyroid follicular epithelial cell-specific deletion of IFT88 gene lacked PC and showed normal hormonogenesis. Older mice showed malignant properties in follicular cells, characteristic of thyroid carcinomas	Lee *et al.* (2019)
	Deletion of IFT88 in thyroid cancer cell lines using CRISPR showed defect in PC assembly but did not promote cell proliferation, migration, and invasion	Lee *et al.* (2018)
	PC were examined in 92 human thyroid tissues encompassing nodular hyperplasia, Hashimoto's thyroiditis, follicular tumor, Hürthle cell tumor, and papillary carcinoma (PTC). PTC samples showed fewer but longer PC, while most Hürthle cells in benign and malignant thyroid samples had fewer PC. Primary Hürthle cell tumors showed increase autophagy, and decreasing autophagy in Hurthle cell carcinoma cell lines restored PC	Lee *et al.* (2016)
Rhabdomyosarcoma (RMS): malignant cancer of skeletal (striated) muscle	PC and Hh signalling need to be dynamic during the differentiation of myoblasts. PC are assembled during the initial stages of myogenic differentiation but disappear as cells progress through myogenesis. Temporal control of ciliogenesis is lost in RMS	Fu *et al.* (2014)
Bladder cancer (BLCA)	Presence of PC was reduced in BLCA tissues when 404 BLCA and 28 adjacent non-cancerous tissues were examined	Du *et al.* (2018)

Table 1 (continued)

Type of cancer	Brief observation and outcome of the study	References
Cholangiocarcinoma (CCA): cancer of cholangiocytes, the epithelial cells lining the biliary tree	Two miRNAs- miR-433 and miR-22 that target HDAC6 were down-regulated in CCA, both in vitro and in vivo. Genetically increasing these miRNAs reduced HDAC6 and also malignant phenotype of CCA cells	Mansini *et al.* (2018)
	CCA cells overexpress HDAC6, and have reduced ciliogenesis. Ablating PC or overexpressing HDAC6 in normal cholangiocyte cells increased HH signalling, proliferation and induced anchorage-independent growth characteristics of CCA. Inhibiting HDAC6 restored PC, decreased cell proliferation and tumor growth in xenograft model	Gradilone *et al.* (2013)
	HDAC6 mediates autophagy in PC (ciliophagy) in CCA. Inhibiting HDAC6 dependent autophagy in CCA cells and in orthotopic rat model of CCA restored PC, decreased cell proliferation, and reduced tumor growth	Peixoto *et al.* (2020)
Chondrosarcoma: cancer of cartilage	Inhibiting HDAC6 decreased the proliferation of chondrosarcoma cells and suppress the invasion capacity of tumor cells	Xiang *et al.* (2017)
	PC were fewer in neoplastic chondrocytes from malignant human chondrosarcomas, compared with chondrocytes from normal articular cartilage. In a mouse model, increasing Gli2 expression and ablating Ift88 led to hyperactivity of HH signalling and increase in chondrosarcoma	Ho *et al.* (2013)
Renal Cell Carcinoma (RCC)	Loss of VHL hyperactivates AurkA. Bexarotene, a small molecule inhibitor was identified, which restored PC assembly in *VHL* mutants by reducing active AurkA, and subsequently reduced the propensity of subcutaneous lesions to develop into tumors in xenograft model of RCC	Chowdhury *et al.* (2018)
	In a mouse model of clear cell RCC with VHL knock out, loss of PC via Kif3a knockout caused cystic lesions formation in kidney, compared to control. Additional knockout of p53 (*Vhl/TP53/Kif3a*) accelerated cystic lesion formation and malignant transition to ccRCC even more rapidly	Guinot *et al.* (2016)
	When VHL is mutated, it usually cause PC loss and renal cysts that are pre-malignant of RCC. PC were predominantly lost across different subtypes of RCC, when 110 patients' tissues were analysed	Basten *et al.* (2013)
Ovarian surface epithelium (OSE) cancer	When ovarian tissues, healthy OSE, and OSE-derived cancer cells were analysed for PC, ovarian cancer cells showed fewer PC, which was not due to growth arrest, and deregulated Hh signalling. Restoring PC by depleting AurkA in OSE-derived cells reduced tumorigenic Hh signalling	Egeberg *et al.* (2012)
Esophageal Squamous Cell Carcinoma (ESCC)	Prdx1 overexpression is associated with cancers. Depletion of Peroxiredoxin 1 (Prdx1) in ESCC cells restores PC, and the tumorigenic potential of these cells were reduced in xenograft assay	Chen *et al.* (2020)

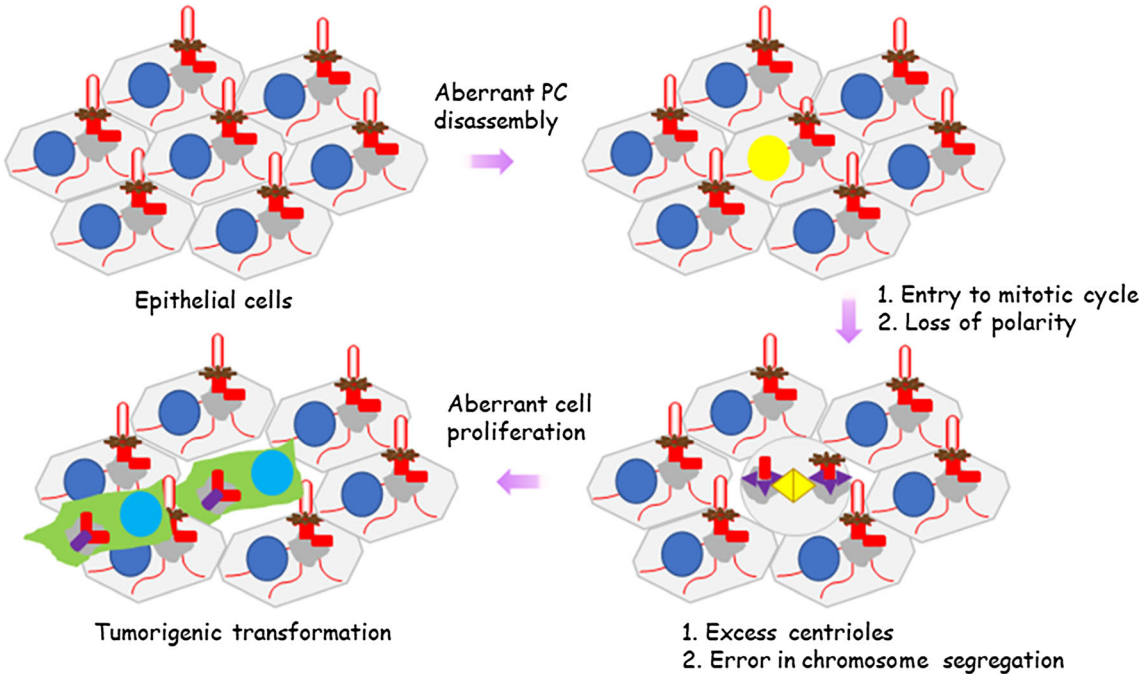

Figure 1. Illustrations showing how aberrant disassembly of the primary cilium (PC) in one of the epithelial cells promoted that cell to enter the mitotic cycle. Additional deregulation in centriole duplication may generate excess centrioles in that cell. Next, that cell undergoes mitosis on a pseudo-bipolar spindle that leads to chromosome segregation error resulting in aneuploidy. Now, cell proliferation generates a bunch of cells that may have lost cell polarity, and acquire the ability to override cell cycle checkpoints, motility and tissue invasion ability, thereby initiating tumorigenic transformation in that tissue.

growth (Khan *et al.* 2016), offering promise and thoughts for future therapeutic intervention against some cancers. Importantly, several negative regulators of PC disassembly (such as- AurkA, Mps1, Plk1, Nek2, CP110, etc.) also promote centriole reduplication by independent mechanisms leading to excess centrioles and/or centrosome amplification, which are common in various cancers. As elegantly demonstrated by Ganem et al, excess centrioles often lead to pseudo-bipolar spindle and merotelic attachment, which gives rise to a certain degree of aneuploidy that is advantageous for tumorigenesis (Ganem *et al.* 2009). Thus, deregulation in the activity of those regulatory molecules may not only promote aberrant PC disassembly in cells, but also such unplanned initiation of cell proliferation may be associated with centriole overduplication and chromosome segregation error (schematically shown in Figure 1.). Ultimately, both these events will synergistically promote tumorigenesis. Therefore, such multi-functional molecules are a more potent target for anti-proliferative strategies. Overall, the relationship between proliferation promoting Hh signalling and tumorigenesis is rather complex and dependent on secondary causative mutation(s), presence of PC in those tissues, etc. Accordingly, whether Hh signalling antagonists or inhibitors of PC disassembly inducing factors may be used as anti-cancer treatment should be carefully decided in a context-dependent manner.

9. Conclusions and future perspectives

This review serves the purpose of bringing forth the evolving concept that the cells need to disassemble PC to progress further in the cell cycle. It may remain debatable if PC poses a brake in cell cycle or if the reduction in PC in tumor tissues or tumor-derived cells are due to an effect of the cell cycle. However, the growing body of evidence clearly hints that aberrant disassembly or removal of PC likely promotes proliferation, particularly in cells where proliferation is not driven by the Hh pathway. Possibly, it requires one or more additional oncogenic events for a forced PC disassembly to induce uncontrolled proliferation. Future studies need to identify such oncogenic event(s) that may turn PC disassembly mediated aberrant proliferation into an uncontrollable one which may promote early stage of tumorigenesis. Albeit most of

the discussed studies in this aspect are correlative in nature, few studies conclusively showed PC loss promoted proliferation and drove tumorigenesis. It is tempting to suggest that the strategies to inhibit the molecular pathways that promote PC disassembly may be used in combination with other anti-cancer therapies (such as anti-proliferative, apoptosis-inducing, etc.) to treat the majority of the solid tumors, or at least several epithelial tumors. Importantly, the research in this field is expanding to comprehend the relationship between PC assembly and disassembly, PC-mediated signalling in adult tissues, cell cycle and proliferation. More pharmacological studies with translational data will be needed from diverse cancers to confidently recommend advancement in cancer chemotherapy.

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