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Cell cycle regulation through primary cilium: A long-forgotten story

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Abstract: Protruded from cytomembrane, primary cilium is a widespread cell organelle that can be found in almost all cell types in Mammalia. Because of its comprehensive requirement in various cellular activities and various functions in different organs, primary cilium has been a valuable research area of human pathology research since the turn of the millennium. And the potential application of the interaction between primary cilia and cell cycle regulation may be the most promising direction as many primary cilium-caused diseases are found to be caused by cell cycle dysregulation resulted from primary cilia defects. Therefore, a deep understanding of the interaction between primary cilia and the cell cycle is in great need. Hence in this review, we mainly described how the interaction between primary cilia and cell cycle proceeds and demonstrated three hypotheses raised from much different research. These hypotheses include (1) Primary cilium as a cellular signaling hub to regulate the cell cycle, (2) Primary cilium as a reservoir of cell cycle regulation-related factors, and (3) Primary cilium as a cell cycle checkpoint or a brake. Nonetheless, we also call for more attention on research of interaction between cell cycle and primary cilia and tried to point out some possible research directions for those who are interested.

Abbreviation

DNA:	DeoxyriboNucleic Acid
PDGF:	Platelet Derived Growth Factor
TGF-β:	Transforming Growth Factor-β
Mtor:	Mammalian Target of Rapamycin
GPCRs:	G Protein-Coupled Receptors
PTCH1:	Patched 1
PDGFR:	Platelet-Derived Growth Factor Receptors
5-HTr6:	5-Hydroxytryptamine (serotonin) Receptor 6
SSTR3:	Somatostatin Receptor 3
MCHR1:	Melanin-Concentrating Hormone Receptor 1
MSC:	Mesenchymal Stem Cell
PKD:	Polycystic Kidney Disease
cAMP:	Cyclic Adenosine Monophosphate
STAT:	Signal Transducer and Activator of Transcription
RTK:	Receptor Protein Tyrosine Kinase
SHP2:	Src Homology Phosphotyrosyl Phosphatase 2
PI3K:	Phosphatidyl Inositol 3-OH kinase
Mek1/2:	MAPK Kinase ½
MAPK:	Mitogen-Activated Protein Kinase
Erk1/2:	Extracellular Signal-Regulated Kinase ½
CDK1:	Cyclin-Dependent Kinases 1
CDK2:	Cyclin-Dependent Kinases 2
HEF1:	Human Enhancer of Filamentation 1
HDAC6:	Histone Deacetylase 6
SuFu:	Suppressor of Fused
E2F1:	E2 Promoter Binding Factor 1
IFT:	Intraflagellar Transport
BBS:	Bardet-Biedl Syndrome
ALMS:	Alstrom Syndrome
WNT:	Wingless/Integrated
NLS:	Nuclear Localization Sequence
Jbn:	Jouberin
NDE1:	Nuclear distribution protein nudE homolog 1
Nek2:	NIMA Related Kinase 2
NIMA:	Never in Mitosis Gene A
DYNLT1:	Dynein Light Chain Tctex-Type 1
IGF:	Insulin-Like Growth Factors
LC8:	Light Chain 8
ESCs:	Embryo Stem Cells
CPAP:	Centrosomal-P4.1-Associated Protein
NPCs:	Non-Parenchymal Cells
MB:	Medulloblastoma

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Introduction

Cilium is a widespread organelle that is mainly consisted of microtubules and locating on the cytomembrane. Its discovery can be traced back to almost 350 years ago by Anthony van Leeuwenhoek (May-Simera *et al.*, 2017). The related research on cilia has already started since the nineteenth century, but there is no significant breakthrough because of the limitations on experimental technologies. Not until the invention and wide application of the electron microscope did scientists begin to figure out the structure and function of the cilium.

Cilia can be divided into two major categories: motile-cilia (or so-called secondary cilia or flagella) and primary-cilia. Motile-cilia, as the name suggests, are capable of wobbling and thus can be found in many bio-tracts with transportation functions, such as the digestive and reproductive tracts. While primary cilia are unable to spontaneously move and thus have been considered as a vestigial version of motile-cilia in higher animal cells. Most of the research has been only focusing on motile cilia for a long period of time. However, since a bunch of unexpected functions of primary cilia was uncovered in the 1990s (Wheatley, 1995), more and more focus has begun to shift to primary cilia. And this passion reached a new level after the turn of the millennium when researchers noticed the correlation between primary cilia and a wide range of human diseases.

Primary cilia were firstly named by Sorokin (1968). And it widely exists in almost all vertebrate cells (Bangs *et al.*, 2015). Primary cilia now have been discovered to have multiple biological functions such as biosensor, cellular signaling transducing hub, differentiation regulator and cell cycle controller et al. Take one for instance, as the bio-sensor, primary cilia have an essential role in olfaction (Tadenev *et al.*, 2011) and optesthesia (Ramamurthy and Cayouette, 2009). Their defects usually lead to a wide range of diseases which are defined as "Ciliopathy" (Reiter and Leroux, 2017).

The structure of primary cilia has been very clear. They have the classical 9+0 structure, which contains nine doublet microtubules but without the central pair of singlet microtubules that often appear inside motile cilia. Primary cilia are unable to move by themselves since molecular motors, and axonemal dyneins are missing (Satir and Christensen, 2007). In general, the structure of primary cilia can be divided into four parts: Basal body, transition zone, axoneme, and the ciliary membrane (Elliott and Brugmann, 2019). However, it is worth noting that there is a special type of primary cilia, nodel cilia, which are capable of oscillating and playing an important role in the determination of the left and right body axes during embryonic development.

Cell cycle regulation is the most important factor influencing cell proliferation, and different cell cycle checkpoints strictly regulate the cell cycle process to ensure normal cell proliferation. In addition to the replication of genetic material during the cell cycle, cell proliferation also involves the replication of centrioles to form a spindle, which is responsible for evenly distributing the replicated genetic material to the daughter cells. As centrioles are also indispensable components for ciliation, it is supposed to have a dynamic and reciprocal interaction between primary cilia and cell cycle as shown in Fig. 1. Tucker and colleagues firstly found the mutually exclusive relation between ciliation and cell proliferation (Tucker *et al.*, 1979). And further research unveiled more this kind of mutually exclusive connections (Breslin *et al.*, 2014). The cell cycle is artificially divided into four phases: G1 (G0), S, G2 and M for research purposes. In most cells, primary cilia start to form during the G1 phase or the early stage of the G0 phase (Nigg and Stearns, 2011). At this point, mitosis just right finishes, and the mother centriole (the older centriole in two centrioles in the daughter cell) is released from the centrosome, an essential component in the spindle apparatus. The released centriole next relocates to the cytoplasmic membrane. Then it recruits appendages to form a basal body, which is the base of the primary cilium (Kobayashi and Dynlacht, 2011). Throughout the whole G0 phase, the primary cilia play important roles to regulate cellular activities. However, this quiescent state begins to transform when the cell prepares to re-enter into a new cell cycle at G0/G1 phase. Firstly, primary cilia initiate their disassembly during G1/S transition under the manipulation of specific proteins (like Aurora-A). Then the mother centriole in the basal body is released. Just like the reverse version of ciliogenesis, the released centriole next relocates to the nucleus and forms the centrosome at the G2/M phase to facilitate the even distribution of genetic material into

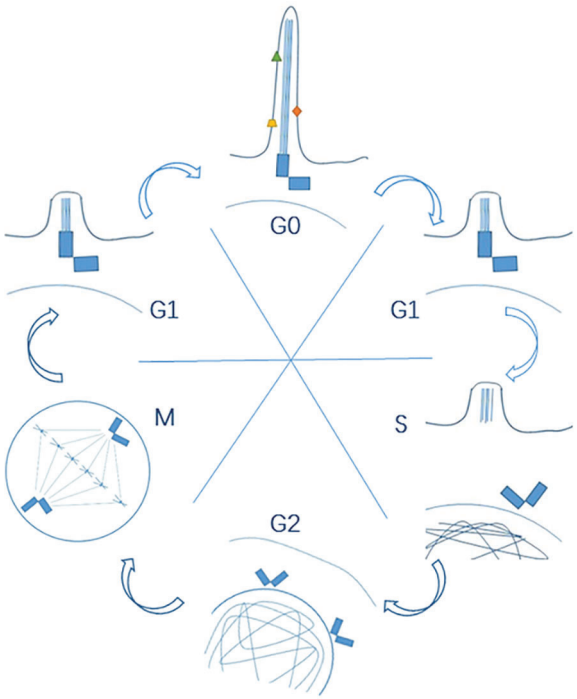

FIGURE 1. A dynamic and reciprocal interaction between primary cilia and cell cycle.
Most cells initiate their ciliogenesis at G1/G0 phase. And at the G1/S transition, they initiate ciliary resorption, and the basal body would release centriole to the cytoplasm. Relocated centrioles become centrosome and help the DNA material evenly distribute into two daughter cells. In each daughter cell, the older centriole would move to the cytomembrane and transform to the basal body again, waiting for the next cell cycle.

two daughter cells during mitosis (M phase) (Liang *et al.*, 2016) (Plotnikova *et al.*, 2009). And after mitosis, the cell enters the G1 phase again and waits for the next cell cycle.

Thus, it is almost an instinct to propose an intimate relationship between the cell cycle and primary cilia as they share a common component. Therefore, what kind of pivotal connection between the cell cycle and cilia becomes an issue worth pondering. Up to now, no reciprocal relation between motile-cilia and cell cycle regulation has ever been reported because motile cilia usually emerge in limited highly specialized cell types, which exit from the cell cycle and undergo no proliferation, to perform specific functions. So, in this review, we only focus on the interaction between primary cilia and cell cycle regulation based on the universal presence of primary cilia.

In addition to these obviously mutually exclusive functions of centriole in both cell statuses, many other studies have also shown more aspects of the connection between cilia and cell-cycle at the beginning of this century (Quarmby and Parker, 2005) and unraveled more intricate interactions between cilia and cell cycle. For example, research also revealed a suppressing effect of primary cilia on cell proliferation in zebrafish embryos; longer primary cilia suppress cell proliferation (Kim *et al.*, 2011). And proliferating tumor cells are considered to be cilium-free. However, a recent study has found a great proportion of tumor cells, such as HeLa and MG63, having primary cilia (Kowal and Falk, 2015). This discovery makes the possible role that primary cilia may play during tumorigenesis or tumor cell maintenance even more obscure but also more intriguing. It should point out that there could be a more sophisticated interaction between cilia and the cell cycle than we usually thought as only the mutually exclusive relation, which also leads more scientists to focus on a deep story about their interaction.

However, the exact mechanism of this interaction is still obscure after decades of research. So, in this review, we summarize the latest literature about the relationship between primary cilia and cell cycle control and put forward three major hypotheses of how the interaction between cilia and cell cycle proceeds. In the end, we also raise up possible problems and research directions to share with research communities and trigger new promotion on this long-ignored area.

Primary cilium as cellular signal hub to regulate cell cycle

Primary cilia were first discovered as an important bridge of signal transduction between the extracellular environment and intracellular activities in 2003 (Huangfu *et al.*, 2003). After that, more signaling pathways have been found to have primary cilia as their signaling hubs or transfer stations (Pala *et al.*, 2017). Downstream cellular activities based on primary cilia-related signaling networks include cell differentiation, proliferation, survival, metabolism, migration, and cell cycle regulation. Thus, impairment of primary cilia can cause defects in signal transduction between the extracellular environment and intercellular signals. And that defects usually result in physiological imperfections (Pruski *et al.*, 2019). It is worth mentioning that many physiological imperfections were caused by cell cycle regulation defects resulted from cilia-related signaling pathway impairments. Under normal physiological conditions, cellular proliferation is under strict regulation through cilia-involved signaling pathways. Therefore the imperfection of cilia-involved signaling pathways caused by defects of cilia might lead to cell cycle dysregulation and then lead to ciliopathies and cancers (Nishimura *et al.*, 2019). While a lot of signaling pathways have been found to be primary cilia-related, many of these signaling pathways lack sufficient research. Therefore, in order to make a general impression about the hypothesis on cilia and cell cycle-related signaling pathways only three relatively in-depth researched primary cilia receptors are demonstrated below. The basic information of other cilia-related signaling pathways is shown in Tab. 1.

The general content of three typical cilia-related signaling pathways is shown in the figure above. (1) Polycystin1/2 regulate gene expression by increasing the intracellular Ca^{2+} concentration to promote the interaction between Ga^{2+} and Calmodulins. (2) Hedgehog signaling (PTCH1) has Gli2 as a transcription factor to do this job. (3) PDGFR initiates cell cycle-related gene expression by recruiting SH2/PTB domain-containing adaptors.

In the table above, it is clearly demonstrated that most of the cilia-related signaling pathways are involved in cell proliferation. And any defects of these signaling pathways or cilia are capable of triggering diseases caused by dysregulation of cell cycle regulation, such as tumors, PKD, or cysts (Pala *et al.*, 2017; Wheway *et al.*, 2018; Labour *et al.*, 2016; Tian *et al.*, 2019; Siebel and Lendahl, 2017; Ibraghimov-Beskrovnaya and Natoli, 2011; Liu *et al.*, 2019; Aspera-Werz *et al.*, 2019).

Polycystin1/2 receptor complex mediated Ca^{2+} pathway

PKD is a common kidney disease characterized mainly by the growth of large and fluid-filled cysts (Bergmann, 2019). It is already known that PKD is caused at least in part by the dysregulated renal cell proliferation. Polycystin1/2 encoded by *PKD1* and *PKD2* (Cornec-Le Gall *et al.*, 2019) respectively is the essential mediator of this process. These two receptors are locating in primary cilia in a form of complex to act as a type of mechanical sensor and ion channel, which in response to extracellular stimuli pump extracellular Ca^{2+} into the cytoplasm. The elevated intracellular Ca^{2+} lets the Ca^{2+} reservoir release more Ca^{2+} (Nauli *et al.*, 2003). For example, in mouse embryonic renal cells, the fluid flow or so-called mechanical stimuli directly act on polycystin-1, and which then triggers polycystin-2 to pump extracellular Ca^{2+} into the primary cilia of the renal cells. Elevated intracellular Ca^{2+} level further stimulates the endoplasmic reticulum membrane to release more Ca^{2+}. Combined with the released Ca^{2+}, the downstream Calmodulins are then activated and keep the renal cell proliferate in a low and normal mitotic index. High-level Ca^{2+} promotes cyclic nucleotide catabolism and makes less cAMP, which suppresses cell proliferation as feedback (Nauli *et al.*, 2003) (illustrated in Fig. 2). If polycystin-1 or polycystin-2 lose their functions resulting from ciliary defects and lead to no extracellular stimuli detection, it will cause low Ca^{2+} concentration and decrease of cyclic nucleotide catabolism inside renal cells. And those two

TABLE 1

Multiple cilia-related signaling pathways and their basic information

Signaling Pathways	Receptors on Cilia	Physiological Roles	Ciliopathies or Diseases
Hedgehog	PTCH1	Proliferation	Tumor
PDGF	PDGFR	Proliferation	Tumor
Ga^{2+} pathway	Polycystin1/2	Proliferation	Polycystic Kidney Disease
TGF-β	TGFβ1/2	MSC* Recruitment	Orthopedic disease
mTOR	Growth Factor Receptors	Cell growth & Proliferation	Renal cysts, Cancer
GPCRs	5-HTr6, SSTR3, MCHR1	Ciliary length	Developmental defects
Notch	Notch3	Proliferation	Cadasil

Note: *Mesenchymal stem cell.

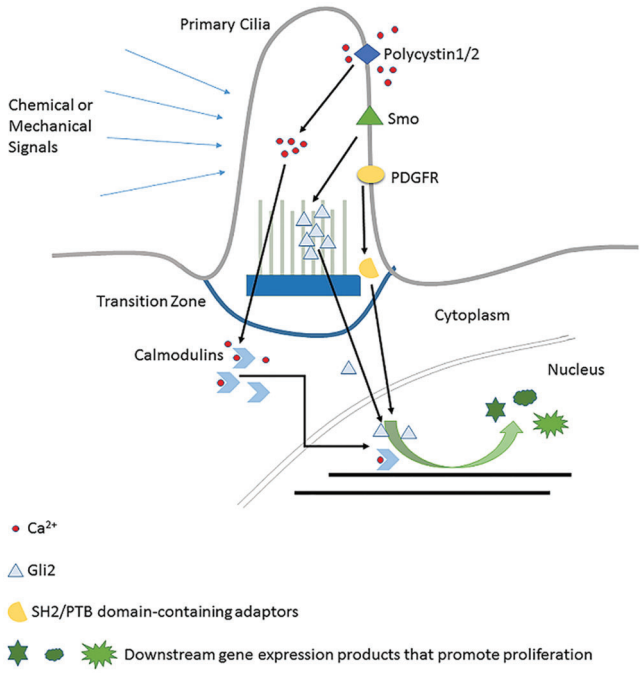

FIGURE 2. Details of three typical cilia-related signaling pathways.

alterations together cause activation of Ca^{2+} inhibitable adenyl cyclase 5/6 and accumulation of cAMP levels, which might promote cell cycle progress pathways (Delling *et al.*, 2013). As a result, renal cells proliferate in a higher mitotic index and finally lead to cystic tissue (Lee *et al.*, 2011). Certainly, another Ca^{2+}-independent gene expression regulation mechanism is also possible, by which polycystin-1 undergoes proteolytic cleavage and its cytoplasmic tail translocate to the nucleus and interact with P100 or STAT to affect downstream gene expression (Low *et al.*, 2006).

PDGF/PDGFR related RTK pathway

PDGFR (Platelet-Derived Growth Factor Receptor) is a widely expressed RTK family receptor locating on primary cilia and reported to transmit extracellular information into the nucleus and regulate the cell proliferation in fibroblast cells (Christensen *et al.*, 2008; Schneider *et al.*, 2005). Autocrine activation of PDGF signaling is involved in certain tumors like gliomas, sarcomas, and leukemia (Andrae *et al.*, 2008). The ligands firstly bind to PDGFR on cilia which let the intracellular tyrosine residues be autophosphorylated. Conformation change allows the recruitment of SH2/PTB domain-containing adaptors, such as SHP2 (Src Homology Phosphotyrosyl Phosphatase 2) and PI3K (Phosphatidyl Inositol 3-OH kinase) to bind. Subsequently, through the Ras-Mek1/2-Erk1/2 pathway, CDK1 and CDK2 are activated to promote cell cycle re-entry (see Fig. 2). This pathway will only be activated when the cell is in the ciliated quiescent status (Schneider *et al.*, 2005). So, it is clear that primary cilia play its essential role in the PDGF signaling pathway. It is noteworthy that this pathway has crosstalk with the HEF1/Aurora A/HDAC6 pathway (Nielsen *et al.*, 2015), which is a very important cilia status regulator. In summary, as an essential cell cycle control signaling pathway, the fulfilling of functions of the PDGF signaling pathway heavily relies on normal primary cilia.

Hedgehog signaling

Hedgehog signaling is an essential developmental pathway. It is also a crucial regulator of cell cycle regulation (Agathocleous *et al.*, 2007; Lupu *et al.*, 2018) and is involved in several diseases caused by abnormal cell cycle regulation. Thus Hedgehog has become a hot spot of tumorigenesis and cancer therapy research (Skoda *et al.*, 2018). The primary cilium is the major site where Hedgehog signaling transduction happens.

Mutual repulsive phenomena between Ptc1 and Smo on the cilium defines different activity states of Hedgehog signaling. In the absence of ligands, the Ptc1 receptor is located on the primary cilia and repress Smo to transport onto cilium. Gli transcription factors, the downstream executant, are thus trapped at the tip of the primary cilium and suppressed by Suppressor of Fused (SuFu). When ligands bind on, Ptc is transferred to the cytomembrane, which lets Smo move onto the ciliary membrane to repress SuFu, removing repression on Gli. Gli activation finally initiates transcription of proliferation-related genes such as Cyclin D, Cyclin E, and E2F1 (Kasper *et al.*, 2006).

It is worth emphasizing that the role of primary cilia in the Hedgehog pathway is far beyond the fact that primary cilia are the location of the receptors. The exact influence of primary cilia on Hedgehog signaling is complicated. Whether these act positively or negatively is context-dependent and also determined by appropriate coordination of ciliary proteins (Wong and Reiter, 2008; Dhekne *et al.*,

2018; Kilander *et al.*, 2018), especially the Intraflagellar Transport (IFT) family. Cilium enclosed space is relatively independent of the cytoplasm. The material and information exchange between them is carried out by the IFT System (Lechtreck, 2015). For the Hedgehog signaling pathway, the Gli2 move from the tip of cilia and then is released to the cytoplasm to help relocate β-catenin (Liem *et al.*, 2012) is also IFT dependent (see Fig. 2). Thus, IFT defects can interfere with Hedgehog signaling function in cell cycle regulation (Wu *et al.*, 2017). And this is a different situation compared with the PDGFR pathway and PKD-related pathway, whose signaling transductions are not IFT-dependent. So, from this point of perspective, Hedgehog might be the most "ciliary pathway" among all the other signaling pathways related to primary cilia.

In summary, primary cilia are associated with numerous pathways that are well known to form complex signaling networks (Christensen *et al.*, 2017). And any defects of these complex signaling networks are about to cause a wide arrange of human diseases, such as cancers, ciliopathies, and obesity *et al.* Among them, the relationship between cancer and primary cilia is the most noticeable. Unregulated cell proliferation is one of the major characters of cancer cells. And as discussed above, primary cilium is a signal hub and many primary cilia mediated signaling pathways are cell cycle regulation-related. Except for the Hedgehog pathway, PDGF pathway, Ga^{2+} pathway mentioned above, more pathways are listed in Tab. 1. Therefore, any defects of primary cilia can give rise to cell cycle regulation defects then lead to cancer. It has been observed that many cancer cells are either non-ciliated (Cao and Zhong, 2016) or those ciliated cancer cells have abnormal primary cilia (Yasar *et al.*, 2017). This phenomenon suggests that the ciliary loss or abnormality may cause cancers. Thus, in Tab. 1, it is intuitive to see that the diseases resulted from primary cilia mediated signal defects are mostly cancers or tumors. Furthermore, some primary cilia-mediated signaling pathway defects only induce mild cell cycle dysregulation as the mentioned PKD and many other ciliopathies with cyst (Tsang *et al.*, 2018). In addition, defects of primary cilia mediated pathways also cause obesity. Obesity is a common character of Bardet-Biedl Syndrome (BBS) and Alstrom Syndrome (ALMS), which are both ciliopathies (Vaisse *et al.*, 2017). This phenomenon indicates a potential intimacy between obesity (energy homeostasis of cells) and primary cilia. However, the molecular mechanism about how exactly defects in primary cilia interact with the energy homeostasis of cells is still unknown. Based on the recent literature, the interaction can be divided into two categories as far: Leptin-dependent and leptin-independent (Vaisse *et al.*, 2017; Volta and Gerdes, 2017). These two pathways are different in molecule details from each other, but both of them are depending on the role of primary cilia as the signaling hubs in cells as many receptors of cellular energy homeostasis related signaling pathways are located in primary cilia, such as lepRb, Sstr3, 5HT6 and Npy2r et al. Therefore, any defects happening to primary cilia can damage the energy homeostasis and cause obesity in individual level.

Generally speaking, the multiplicity of the ciliary receptors system opens up a whole realm of possibilities for the primary cilium to coordinate the cross-talking between different signaling pathways, which in a concerted action, balances the biological output. And therefore, comprehensively regulates the physiological activities of cells, more than just cell cycle re-entry. Recent research shows that in keratinocytes, corneal epithelia and neuroepithelia, primary cilia are able to regulate cell proliferation and differentiation by regulating the Notch signaling pathway (Wheway *et al.*, 2018). Although the mechanism is still intriguing, the implication of coordination from different pathways illustrates again the complicacy.

Primary cilium as a reservoir of cell cycle regulation factors

Primary cilia is a cell cycle regulation related factor reservoir structurally and compositionally separated from cytomembrane and cytoplasm (Satir and Christensen, 2007). When cilia experience physiological resorption or pathological structure damage, transcription factors or signaling pathway members originally stored inside primary cilia might be released to the cytoplasm and even end up being inside the nucleus. Gene expression profile regulating the cell cycle control may be changed. This mechanism is usually described as the "Reservoir Hypothesis" (Kim and Tsiokas, 2011) (see Fig. 3). Jouberin (Jbn) (Lancaster *et al.*, 2011) and Gli2 (Han *et al.*, 2009) are two typical cases of this hypothesis.

Jbn is encoded by AHI1, mutations of which mainly contribute to the Joubert Syndrome (Dixon-Salazar *et al.*, 2004). The β-catenin is the core component of the canonical WNT pathway. Extracellular signals received by Wnt receptors (Frizzled, Lrp) are transmitted into the nucleus to regulate the downstream cellular activities including cell cycle re-entry, during which the nuclear localization of β-catenin is the most critical step. However, β-catenin itself has no nuclear localization sequence (NLS) responsible for this trans-localization. Jouberin (Jbn) is one kind of protein facilitating β-catenin's re-localization and accumulation inside the nucleus (Lancaster *et al.*, 2009). Jbn usually locates inside of primary cilia (Lancaster *et al.*, 2011). However, this isolation status can be broken up under some conditions and the molecules are transferred out by the ciliary IFT system (Lancaster *et al.*, 2009; Lancaster *et al.*, 2011). So when cells are in quiescent status, primary cilia act as a negative regulator of Wnt signal to keep Jbn inside and away from β-catenin (Lancaster *et al.*, 2011). While when the primary cilia experience resorption, Jbn may be released to the cytoplasm but in an IFT-independent way and promote Wnt-signaling by transferring more β-catenin into the nucleus. Then β-catenin makes proliferation-related genes express and finally leads to cell cycle re-entry (shown in Fig. 3).

As mentioned above, activation of Hedgehog signaling leads to the release of Gli factors to the cytoplasm, which are originally restricted inside primary cilium by SuFu in quiescent cells. Thus, those sequestered Gli factors (such as Gli2) use primary cilium as the reservoir. Under normal conditions, Gli2 could be released to the cytoplasm by Hedgehog ligands binding on Ptc1 to relieve Smo's repression on SuFu. However, in some cases when primary cilia experience resorption (Han *et al.*, 2009; Wong *et al.*, 2009; Zhao *et al.*, 2017), Gli2 could also be released to the

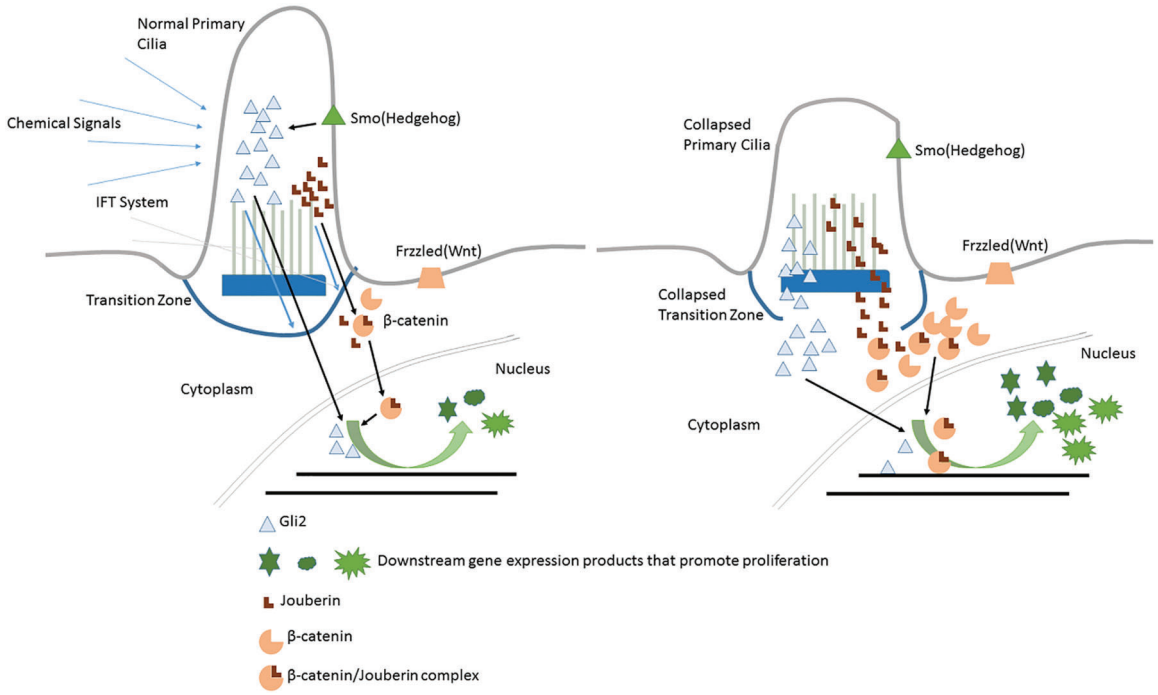

FIGURE 3. Primary cilium as a reservoir of cell cycle regulatory factors.
The left picture shows the primary cilia under normal physiological conditions. Its structure and function are complete, and transcription factors (Gli2 and Jbn) are sequestered inside primary cilia. Their transmission is under strict regulation (the IFT system is responsible for this regulation, shown in the blue arrow inside primary cilia in the left). However, if the structure is defective, sequestered factors would release from the primary cilia without any regulation (without the IFT system being involved in the right model) and cause dysregulated transcription factor transmission. And finally, it would lead to violent cell proliferation. This violent cell proliferation is responsible for many ciliopathies and tumors.

cytoplasm, which makes the higher concentration of cytoplasmic Gli2. Excess Gli2 will be transferred into the nucleus and activate cell cycle promotion genes, leading to cell cycle dysregulation, which causes ciliopathies or tumors.

In conclusion, Jbn and Gli2 are originally sequestered inside primary cilia. When primary cilia are experiencing abnormal resorption or collapse, Jbn and Gli2 would be released into the cytoplasm and over-activate cell cycle-related signaling pathways. This hypothesis is different from the one that primary cilia are the cellular signal hub. Because there are no extracellular stimuli nor complete signal transduction involved. And this field calls for more attention.

Primary cilium as a cell cycle checkpoint or brake

Cell cycle checkpoints determine whether and when do cells enter the cell cycle. Many cellular events have been considered as checkpoints during the cell cycle. In fact, many characters of the interaction between primary cilia and cell cycle meet the features of being considered as a cell cycle checkpoint. First, when proteins promoting ciliogenesis such as NDE1 (Kim *et al.*, 2011) and Tctex-1 (Li *et al.*, 2011) are overexpressed, cells will experience cell cycle arrest. Second, when proteins suppressing the ciliogenesis such as Aurora-A and trichoplein are inhibited, the cell will experience cell cycle arrest as well (Zhu *et al.*, 2017). And third, manipulating IFT proteins to make cell compulsorily experience ciliogenesis or ciliary resorption can reverse the effects by all the molecules. These three facts imply a similarity of primary cilia with the pattern of general checkpoint. Therefore, primary cilia might work as a physical checkpoint before cell cycle re-entry (Goto *et al.*, 2017). This is the checkpoint hypothesis. In other words, by manipulating the length of primary cilia or the timing of ciliogenesis, researchers are able to delay the cell cycle re-entry or initiate it in advance (Hsiao *et al.*, 2018). Many proteins functioning as ciliary regulators have been involved in this hypothesis. Such as Nde1, Tctex-1, Aurora-A-HEF1, Kif24 (Kim *et al.*, 2015), Pifo (Kinzel *et al.*, 2010), and IFT88 and IFT27 (Qin *et al.*, 2007; Robert *et al.*, 2007).

1. Aurora-A

Aurora/Ipl1-related kinases are evolutionally conserved serine/threonine kinases (Marumoto *et al.*, 2005). And it is a key regulator during oncogenesis and a potential cancer therapeutic molecule (Katayama *et al.*, 2003; Yan *et al.*, 2016). Aurora-A influences the cell cycle by making the balance between ciliary resorption and ciliary disassembly towards disassembly (Izawa *et al.*, 2015; Kasahara *et al.*, 2018). After ciliary disassembly caused by Aurora-A, the cell would initiate its cell cycle re-entry process. Furthermore, Aurora-A is also capable of suppressing ciliogenesis during cell mitosis (Goto *et al.*, 2017; Inaba *et al.*, 2016). This demonstrates the dual function of Aurora-A in regulating mitosis. And it is a clear truth that being ciliated or not is a key feature before the cell cycle re-entry.

2. Nek2

Nek2 is an S/G phase kinase, which consists of two subtypes, Nek2A and Nek2b (Pfleger and Kirschner, 2000; Hames *et al.*, 2001), and it has been found to function as an oncogene in many cancers (Cappello *et al.*, 2014; Zhou *et al.*, 2013; Hayward *et al.*, 2004). There are researches

demonstrating Nek2 as a suppressing factor of ciliogenesis (Spalluto *et al*., 2012; Kim *et al*., 2015), in which Nek2 and Kif24 work together to suppress ciliogenesis and promote ciliary disassembly and, next, cells are allowed to initiate cell cycle re-entry (Kobayashi *et al*., 2011). This phenomenon is consistent with the one described Aurora-A that when cilia are removed, cells would initiate the cell cycle re-entry.

3. Tctex-1

Tctex-1, also known as DYNLT1, is a dynein light chain protein. It is mostly discovered in neural precursor cells and plays an important role in neuron differentiation. When precursor cells lose its ability to proliferate, it begins to differentiate (Gauthier-Fisher *et al*., 2009). This fact suggests a possible interaction between Tctex-1 and cell cycle re-entry. And later research found that only in ciliated cells does Tctex-1 have an impact on G1/S transition (Li *et al*., 2011), demonstrating an essential role of cilia in the cell cycle. A further mechanism was uncovered that Thr96 Phosphorylated Tctex-1 can cause cilia resorption by IGF-1 and promote cell cycle re-entry (Yeh *et al*., 2013). However, only in ciliated cells can Tctex-1 be phosphorylated in Thr94 (Li *et al*., 2011). So phosphorylated Tctex-1 promotes ciliary resorption in a cilia-dependent way and leads to cell cycle re-entry. In summary, primary cilium is a cell cycle checkpoint and functions through Tctex-1.

4. Nde1

Nde1, interacting with dynein light chain LC8, can negatively regulate the ciliary length. It expresses at a high level during the M phase while at a low level during the G0 phase. And depletion of Nde1 leads to longer primary cilia delay in G0/G1 transition (Kim *et al*., 2011). Those two facts together suggest a connection between Nde1 and cell cycle regulation through cilia length. By knockdown Nde1 in zebrafish embryos, cells are found with longer primary cilia but with a lower mitosis index (Kim *et al*., 2011). So, Nde1 regulates cell cycle re-entry timing by controlling the length of primary cilia.

What is worth emphasizing here is that primary cilia play an essential role as the so-called checkpoint of mitosis not because of the dual functions of those proteins in both ciliogenesis/resorption and cell cycle re-entry and also not simply because both mitosis and ciliogenesis require the involvement of centriole. The quintessence of this hypothesis here is that cilia are considered as a checkpoint before and during cell cycle re-entry, just like other checkpoint parameters: Cell size, environmental nutrition, and DNA integrity. This feature is well demonstrated in Tctex-1 and Nde1: The presence or the length of cilia both influence the timing of cell cycle re-entry, just like the biological effects of other cellular checkpoint factors. Take DAN reparation, for instance, damaged DNA material without appropriate reparation leads to proliferation arrest (Karimian *et al*., 2016). And initiating ciliogenesis during mitosis also leads to proliferation arrest. Moreover, almost all the mentioned molecules (Aurora A, Nek1, Tctex-1, and Nde1) are oncogenesis-related. This supports the "checkpoint hypothesis" in an indirect way. Because many other cell cycle checkpoints related factors are also oncogenesis-related. It is easy to understand. These checkpoints molecules are ciliogenesis or ciliary resorption related. If they lost their functions, cells might be able to re-enter the cell cycle without appreciating regulation. Thus, all these proteins naturally can be considered as proto-oncogenes and tumor suppressor genes and should be brought to the light and considered as potentially promising cancer treatment targets.

Primary cilia and embryonic stem cell maintaining and differentiation

Regulating differentiation can be considered as a special form of cell cycle regulation. Because when a cell specializes, it loses the ability to divide while the cell non-specialized keeps its ability to divide. Primary cilia can also be discovered in embryonic stem cells (ESCs). Combining the following facts: most of the differentiated cells lost their ability to divide and primary cilia regulate the cell cycle, it is logical to assume that primary cilia may regulate cell differentiation as well. Literally, there is increasing evidence indicating that primary cilia may play an important role in ESCs maintaining and differentiation or the so-called asymmetric division (asymmetric division decide the fate of the daughter cells). Take Tctex-1, for instance, as mentioned above, it is an important neuron differentiation factor. And the possible mechanism of Tctex-1 promoting differentiation is suppressing Tctex-1 to keep cells ciliated. And these ciliated cells would lose their ability to divide and begin to differentiate. CPAP works the same way. In *in vitro* experiments, when CPAP is mutated, it fails to negatively regulate cilia length. This causes long cilia, retarded ciliary disassembly, and delayed cell cycle re-entry, and further leads to premature differentiation of NPCs (Gabriel *et al*., 2016). This is the first way of cilia regulating cell fate. Second, many embryonic stem cell makers are discovered on primary cilia in ESCs, including Oct4, Sox2, and Nanog. These factors are essential for stem cell maintenance. So this fact indicates a possibly critical role that primary cilia may play in ESCs maintaining (Vestergaard *et al*., 2014), but the mechanism is not known yet. Third, as the signaling hubs in cells, many signaling pathways are primary cilia-dependent. And these primary cilia-dependent signaling pathways are playing a significant role in not only cell cycle re-entry regulation but also ESCs maintaining or differentiation; these signaling pathways include PDGFR (Pébay *et al*., 2005), Hedgehog (Hunkapiller *et al*., 2011), and Wnt/β-catenin (Sato *et al*., 2003), and so on. Thus, by regulating the signal transduction, primary cilia become a key part of the cell differentiation manipulation center. In summary, Primary cilia regulate ESCs, both stem maintaining and differentiation, in three different ways.

Conclusion

In this review, three hypotheses were demonstrated in detail: (1) Primary cilium as a cellular signaling hub to regulate the expression of cell cycle control-related genes, (2) Primary cilium as a reservoir of cell cycle regulation-related transcription factors, (3) Primary cilium as a cell cycle brake or checking-point. However, this forgotten field has not been given enough attention. In terms of the first hypothesis, no further interaction has been uncovered so

far. It is not clear whether primary cilia can regulate signaling pathways in a way more than just the scaffold of signaling, whether there is molecular cross-talking among different signaling pathways through or coordinated by primary cilia, and whether primary cilia can regulate signal strength and select downstream targets so to have different impacts on cell cycle. All these problems are attractive and need more attention. In the second hypothesis, the research of the molecular mechanism of Medulloblastoma genesis (MB) is the major resource on the information of Gli2 as a cilia-sequestered factor inside primary cilia so far. But there still is no direct evidence to prove that Gli2 or Jbn literally function in a way that perfectly matches the pattern of the hypothesis. And Medulloblastoma is still a promising information resource for future research so far. In the third hypothesis, it is clear to see the potential of primary cilia as a brake of cell cycle control. And these molecules mentioned above are all possible counterparts of other mature proliferation brake components such as p53 and p21. All those proteins (Aurora-A, Nek2, Tctex1, and Nde1) are promising targets for tumor treatment and require deeper research (Chen *et al*., 2020). And making cilia-free tumor cells ciliated may be a good way to suppress tumor spread and finally leads to complete cure. And as almost all human cells are ciliated, we would not see any potential of negative side effects. In the end, we briefly described the relation between primary cilia and stem cell maintaining/differentiation, which is similar to the pattern of interaction between the cell cycle and primary cilia. Therefore, there may be a more sophisticated interaction among these three cellular activities (ciliation, proliferation, and stem cell maintaining/differentiation).

From something being thought of as a vestigial structure in cells to a cellular organelle being considered as one of the most valuable research fields, the research on primary cilia has encountered its highlight moment in the 21st century, but the light is not bright enough yet. Many promising fields are quite away from the research spotlight, such as the interaction among primary cilia, cell cycle regulation, and tumorigenesis. Therefore, we sincerely hope that all this obscure can be clarified in future researches. And all potentials can become realities.

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