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Mechanisms for non-mitotic activation of Aurora-A at cilia

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Abstract

Overexpression of the Aurora-A (AURKA) kinase is oncogenic in many tumors. Many studies of AURKA have focused on activities of this kinase in mitosis, and elucidated the mechanisms by which AURKA activity is induced at the G2/M boundary through interactions with proteins such as TPX2 and NEDD9. These studies have informed the development of small molecule inhibitors of AURKA, of which a number are currently under preclinical and clinical assessment. While the first activities defined for AURKA were its control of centrosomal maturation and organization of the mitotic spindle, an increasing number of studies over the past decade have recognized a separate biological function of AURKA, in controlling disassembly of the primary cilium, a small organelle protruding from the cell surface that serves as a signaling platform. Importantly, these activities require activation of AURKA in early G1, and the mechanisms of activation are much less well defined than those in mitosis. Better understanding of the control of AURKA activity, and the role of AURKA at cilia, are both important in optimizing the efficacy and interpreting potential downstream consequences of AURKA inhibitors in the clinic. We here provide a current overview of proteins and mechanisms that have been defined as activating AURKA in G1, based on study of ciliary disassembly.

Keywords

Cilium; AURKA; serine/threonine kinase; human malignancy; ciliopathy; signal transduction

Introduction

More than 20 years ago, a serine/threonine kinase required for correct spindle formation was identified in *Drosophila* and named “aurora” based on the phenotype of dispersed and asymmetric astral microtubules found in hypomorphic mutants [1]. This was the first characterized member of a kinase family that regulates mitotic events including spindle assembly and cytokinesis, and pre-mitotic events including centrosomal maturation. These activities require the cyclic, strong activation of the kinase in late G2 and its inactivation at mitotic completion [2]. Aurora kinases are evolutionally highly conserved from yeast to

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mammals [3]. The single family member in *Drosophila* has three paralogs in humans - Aurora kinase A (AURKA), which localizes to the centrosome and radial microtubules [4], Aurora kinase B (AURKB), and Aurora kinase C (AURKC) – with AURKA maintaining the centrosomal and spindle functions initially described in flies, and AURKB and AURKC fulfilling other roles [2]. In addition to its role in mitosis, AURKA has been reported to regulate stem cell differentiation, self-renewal and reprogramming [5, 6]. Reflecting these essential roles, oncogenic defects in regulation of AURKA are frequently noted in cancer. AURKA is frequently amplified or overexpressed in numerous malignancies, including but not limiting to breast cancer, leukemia, bladder, ovarian, gastric, esophageal, liver, colorectal, and pancreatic cancers [7-14]. Because of this role in cancer, AURKA is in the focus of development of drugs that block its activation by various mechanisms [4, 15] that have been defined based on studies of the protein in mitosis [16-19].

In contrast to the general focus on AURKA in mitosis, an increasing number of studies have documented an additional role of AURKA. This role reflects non-mitotic functions at the ciliary basal body, a structure that differentiates from the centrosome in non-proliferating, post-mitotic cells [20]. The growing literature on AURKA regulation at cilia suggests additional mechanisms for the control of AURKA activity than those previously identified through studies of AURKA activation in mitosis, which may be relevant to its action in cancer and other pathologic conditions. These alternative mechanisms of AURKA regulation are the focus of this review.

As general introduction, cilia fall into two categories: motile and immotile (or primary) cilia. Motile cilia have many features in common with the flagella of single cell eukaryotes [21], and are found in a limited number of cell types in vertebrates [22]. However, single primary cilia, are present on virtually all cell types in vertebrates (with several notable exceptions, e.g. hematopoietic cell lineage have typically been considered as constitutively non-ciliated, although a recent study has called even this exclusion into question [23]). Primary cilia are expressed from the earliest stages of organismal development, contributing to appropriate pattern formation [24], and are retained in adult tissue. The primary cilium is an organelle organized around a microtubule-based axoneme nucleated at the ciliary basal body, which protrudes from the cell surface and acts as an antenna sensing and transducing various extracellular signals. Although first discovered at the end of 19th century [25], the function of the primary cilium was long obscure, with this structure initially hypothesized to act as a dock for centrosomes in non-mitotic cells [26]. However, over the past two decades, a large number of studies have unveiled the critical regulatory functions for primary cilium, emphasizing its importance in developmental biology and many human diseases.

The primary cilium is now known to provide a spatially concentrated platform for receipt of extracellular cues and induction of intracellular response for signaling pathways downstream of Sonic Hedgehog (SHH) [27], WNT [28, 29], Notch [30], PDGFR α [31], polycystins (PC1 [32] and PC2 [33]) and others. Activity of these pathways depends in large part on the presence or absence of a primary cilium on the cell surface. Some pathogenic conditions, described below, are associated with constitutive changes in cilia, because of dysfunctional structure or chronic loss. However, an intriguing feature of the cilium, long-known but poorly understood, is the fact that it undergoes regular protrusion and resorption that is

coordinated with cell cycle [34]. Typically, cells are ciliated in quiescent G0 and early G1 cells, and invariably, cells lack cilia during mitosis. In most cells, resorption occurs in G1, as cells are induced to active cycling [35]. While the mechanics of the reassembly process have been established to require changes in the action of the intraflagellar transport (IFT) machinery [36, 37], exact details of the disassembly mechanism remain to be elucidated.

In 2007, AURKA was first identified as a proximal regulator of disassembly and resorption of primary cilia [38], an activity paralleling that of its distant orthologue, CALK, in regulating disassembly of motile flagella in the green algae *Chlamydomonas reinhardtii* [39]. As impairments in the assembly, maintenance and disassembly of the primary cilium are positioned to impact all the cilia-dependent signaling networks, these findings spawned intensive subsequent study. As a consequence of this work, many new mechanisms for activation of AURKA in non-mitotic cells have been suggested. These connections may also be extremely relevant to the role of AURKA in cancer and other pathogenic conditions, and suggest additional ways to target the kinase, as we discuss here.

Assembly and Disassembly Control of AURKA activation

Our emphasis in this review is on signaling pathways that impinge directly or indirectly on control of AURKA activity (Figure 1, 2). Proteomic studies of the primary cilium have identified hundreds of proteins associated with this organelle, many of which have been implicated in regulating or mediating the processes of ciliogenesis and ciliary resorption [40-43]. Sanchez and Dynlacht have provided a recent comprehensive review of these processes [44]. Studies addressing the mechanisms regulating assembly and disassembly typically identify Aurora A (AURKA) as a proximal component of the disassembly machinery, activated by interaction with a group of directly interacting partner proteins, and sometimes implicating its interactions with a tubulin deacetylase, HDAC6 [38] (Figure 1). More recently, other components of cilia disassembly mechanism have been defined, including signaling from PLK1 – another kinase best known for function in mitosis – and the microtubule-associated kinesin motor KIF2A [45], the NEK2 kinase with an alternative kinesin, KIF24 [46], and other pathways (e.g., NEK8 [47, 48]). For PLK1 and NEK8, potential mechanisms for crosstalk with AURKA have been shown. The more recently proposed NEK2 pathway has been suggested to be completely independent, although due to limited state of current knowledge, AURKA involvement cannot be completely excluded.

Activation of AURKA during ciliary disassembly

1. AURKA proximal signaling—An unexpected non-mitotic role of AURKA in promoting ciliary disassembly was first shown by Pugacheva et al. [38]. They observed that the levels of T²⁸⁸-auto-phosphorylated AURKA and of its activator, the scaffolding protein NEDD9 [49], significantly increased at 1-2h and 18-24h after stimulating starved, quiescent cells with serum to induce cell cycle re-entry. These activation dynamics strongly correlated with the percentage of ciliated cells; and while the 18-24 hour peak represented G2/M, and a known time of AURKA activation and ciliary resorption, the 1-2 hour peak reflected cells in G1 phase. Moreover, siRNA knockdown or pharmacological inhibition of AURKA completely blocked serum-induced ciliary disassembly, while NEDD9 knockdown greatly

limited disassembly. To provide stronger evidence for a ciliary role of AURKA, they performed microinjections of preactivated wild type AURKA, hypomorphic mutant AURKA, and inactive mutant AURKA into cells, and observed an extremely rapid deciliation with preactivated AURKA, a modest decrease in ciliation for the hypomorphic mutant, and no phenotype with inactive mutant AURKA. This study identified HDAC6 as a candidate downstream effector of AURKA, showing pharmacological inhibition of HDAC6 blocked ciliary disassembly, and finding that microinjection of preactivated AURKA into cells with siRNA-depleted HDAC6 caused delayed and unstable deciliation, with the majority of disassembled cilia reconstituted in 1 hour. They also showed that AURKA interacts with and specifically phosphorylates HDAC6 enhancing its activity. Connecting these data, the authors proposed that upregulation of NEDD9 promoted transient, AURKA autophosphorylation, which allowed AURKA-dependent activation of HDAC6; active HDAC6 deacetylated microtubules in the ciliary axoneme, contributing to its destabilization [38]. Interestingly, a reverse process of polymerized α -tubulin K40 acetylation by the α TAT1 acetyltransferase, a highly conserved protein among organisms assembling cilia, was subsequently shown to significantly promote ciliogenesis [50].

Adding to this initial outline of AURKA regulation, Kinzel et al. determined that mutations in the ciliary gene *Pitchfork (Pifo)* led to ciliary defects and left-right asymmetry abnormalities during development [51]. Pifo, a little-studied small (191 aa) protein with no defined sequence motifs, which has been implicated in Hedgehog signaling [52], colocalizes with AURKA at the ciliary base and directly interacts with AURKA in pulldown assays. Overexpression of Pifo in cells resulted in higher level of phosphorylated AURKA during ciliary disassembly, and a longer duration of AURKA activation. Conversely, overexpression of R80K-mutated Pifo (identified as a heterozygous mutation in two cases of ciliopathy-associated phenotype in human patients [51]) completely abolished AURKA activation while having no impact on protein stability or AURKA interaction. Interestingly, the observed accumulation of Pifo at ciliary base was cell cycle dependent, with the highest levels present during early assembly or disassembly of primary cilia. In addition to enhancing AURKA activity, Pifo also has been found important for preventing centrosome overreplication during S phase; additional roles are under investigation.

Inoko and colleagues reported that Trichoplein (TCHP), originally identified as a keratin filament binding scaffold protein [53], co-localized with AURKA at centrioles, and showed that it interacted with and promoted activation of centriolar AURKA, thus explaining its role as a negative regulator of ciliogenesis [54]. Extending the TCHP signaling network, Inaba et al. showed that NDEL1, an NDE1 paralog, supports trichoplein expression by protecting it from proteasomal degradation, suppressing axonemal extension [55]. NDE1, a member of nuclear distribution E (NudE) protein family, been found to act as a negative regulator of ciliary length, possibly through its interaction with the dynein-containing DYNLL1/LC8, which is associated with retrograde IFT components [56]. In a recent study of radial glia precursors using in vivo shRNA-mediated gene knockdown [57], only depletion of NDE1 significantly increased ciliary length, while knockdown of either NDE1 or NDEL1 resulted in cell cycle block (G₂/M and G₁/S arrest for NDE1 and NDEL1 respectively). Interestingly, double knockdown of these two proteins caused an exacerbated G₁/S block, suggesting

dominance of the NDEL1 phenotype. Whether NDEL1 directly controls TCHP is not yet known.

Beyond NEDD9, other proteins have been proposed to contribute to scaffolding AURKA activation and for ciliary regulation. Centrosomal-P4.1-associated protein (CPAP), mutated in microcephaly-associated Seckel syndrome, was found to interact and possibly serve as a scaffold for a ciliary disassembly complex comprised of NDEL1, AURKA, HDAC6 and another protein, Oral-facial-digital syndrome 1 protein (OFD1) [58]. Neural progenitor cells with CPAP knockdown exhibited excessively long cilia, delayed cell-cycle re-entry and premature differentiation. The mechanism by which CPAP influences AURKA activation has not been investigated.

There is increasing evidence for a role for calcium signaling in regulation of AURKA activation in disassembly of the primary cilium. Plotnikova et al. showed that calmodulin directly interacts with the N-terminus of AURKA in a calcium-dependent manner, resulting in AURKA autophosphorylation of S51, S53/S54, S66/S67 or S98, and demonstrated that this interaction is required for serum-induced cilia disassembly and mitotic progression [59, 60]. Inducible over-expression of S51A-, S53A/S54A-, or S66A/S67A-mutated AURKA derivatives in a wild type AURKA-depleted background failed to restore serum induced ciliary disassembly, in comparison to reconstitution with wild type AURKA. These data add a suggestion of mechanism to an increasing number of studies emphasizing the importance of the AURKA N-terminus, which is required for AURKA centrosomal localization [61], interaction with its activator NEDD9 [49, 62] and regulation of its proteosomal degradation after mitosis [63].

An upstream pathway for calcium signaling as mediator of some stimuli for ciliary resorption was subsequently identified by Nielsen and colleagues [64], who found that PDGF-DD-induced deciliation occurs through direct PDGFR β binding and activation of PLC γ . This triggered calcium release from internal stores and subsequent AURKA activation. In the same study, expression of a constitutively active PDGFR α D842V mutant, which had increased affinity for binding PLC γ instead of the preferred effectors of WT PDGFR α (MEK1/2-ERK1/2 and AKT), led to a significant loss of cilia. This was reversed by pharmacological inhibition of AURKA or PDGFR α , emphasizing the important role of PLC γ in AURKA activation and ciliary resorption. Both these studies [59, 64] confirmed that forced release of calcium from intracellular storage by ionophore ionomycin led to rapid loss of primary cilia.

Plotnikova et al. showed a complex interaction between inositol polyphosphate-5-phosphatase E (INPP5E), a protein defective in some ciliopathies, and AURKA that influenced ciliary resorption. AURKA and INPP5E signaling was bidirectional. INPP5E promoted AURKA autophosphorylation, most likely by producing phosphatidyl inositol-(PtdIns)-(3,4)-diphosphate, which facilitated AURKA activation. Their conclusion was based in part on data indicating that co-expression of INPP4B (a phosphatase decreasing the levels of PtdIns(3,4)P₂) with AURKA and INPP5E in HEK293 cells significantly decreased the level of T²⁸⁸-auto-phosphorylated AURKA. In turn, AURKA phosphorylation of INPP5E increased its phosphatase activity and thus production of PtdIns(3,4)P₂ forming a

short positive feedback loop. More indirectly, increased hydrolysis of $\text{PtdIns}(3,4,5)\text{P}_3$, led to a negative feedback loop that decreased AURKA transcription [65].

Lee and colleagues identified a new signaling cascade for ciliary disassembly in which non-canonical WNT pathway activation caused Plk1-dependent stabilization of the direct AURKA activator NEDD9 [66]. They first identified the WNT-pathway effector Disheveled-2 (Dvl2) as a PLK1-binding protein, and with this interaction mediated by two casein kinases able to phosphorylate Dvl2 to create a binding site for Plk1, CK1 δ and CK1 ϵ . Among the two, only CK1 ϵ knockdown led to impaired ciliary disassembly. Treatment of cells with conditioned media enriched with Wnt5 α , a WNT superfamily member activating non-canonical WNT pathway signaling and CK1 ϵ , significantly increased the levels of phospho-Dvl2 (S143 and T224), increased the formation of Dvl2-Plk1 complexes in a CK1 ϵ -dependent manner. Subsequently, the Dvl2-Plk1 complex bound the NEDD9-interacting protein SMAD3, preventing it from targeting NEDD9 for degradation, and hence indirectly promoting AURKA activation. Extending this signaling axis, Shnitsar et al. showed that the phosphatase PTEN, an extensively studied tumor suppressor [67, 68], also can contribute to ciliary dynamics regulation by dephosphorylating Dvl2 at S143 and thus acting as a negative regulator of the previously described signaling pathway [69].

Besides its interaction with Dvl2, PLK1 has been implicated in interactions with a kinesin, KIF2A, that also converge with AURKA activation control. Miyamoto et al. conducted an extensive study characterizing Plk1-Kif2A pathway promoting ciliary disassembly, in which PLK1 phosphorylation on T544 of Kif2A activated this kinesin to promote microtubule disassembly in vitro, although a detailed mechanism is yet to be elucidated [45, 70]. In separate work, Jang et al. have studied AURKA and PLK1 in mitosis during spindle formation, and have shown these proteins act antagonistically in regulation of Kif2A, with AURKA decreasing depolymerization activity of Kif2A [71]. Whether similar actions occur in feedback during ciliary disassembly, is unknown.

In more indirect regulation of AURKA, Gong et al. have linked Peroxiredoxin 1 (PRDX1), an enzyme that reduces reactive oxygen species, to regulation of the primary cilium [72]. They showed that esophageal squamous cell carcinoma cell lines expressing high levels of peroxiredoxin had suppressed cilia formation, while ciliation was restored by shRNA knockdown of peroxiredoxin shRNA-knockdown. They suggested that peroxiredoxin suppresses cilia formation by activating AURKA through an LKB1/AMPK pathway. LKB1 has been reported to inhibit the AURKA activator NEDD9 [73], and the authors showed that shRNA knockdown of peroxiredoxin 1 led to increased levels of LKB1 and hyper-phosphorylated AMPK, but decreased T²⁸⁸-phosphorylated AURKA levels.

Nek2-Kif24 pathway: The kinase Nek2 also contributes to ciliary disassembly [46]. Nek2 localizes to the proximal region of centriole throughout cell cycle, but preferentially expands localization profile to include the distal region of the mother centriole during the S/G₂ phases of the cell cycle. Nek2 interacts and directly phosphorylates the kinesin Kif24, increasing its microtubule depolymerizing activity, and contributing to ciliary resorption, with most of this activity observed during G₂/M phase [46, 74]. While this mechanism has been validated for pre-mitotic ciliary resorption, it has not been shown to contribute to

G0/G1 resorption events, which have been shown to be regulated by AURKA-HDAC6 and Plk1-Kif2A. Although no intersection between this pathway and AURKA activation controls have yet been described, this pathway was only recently detected, and connections may emerge. For example, in a study by Pugacheva et al. it has been shown that NEDD9 not only is able to activate AURKA but also inhibits NEK2 activity; however, a possible relationship between AURKA and NEK2 was not explored [49].

In the kidney epithelium, formation of primary cilium is regulated by the VHL protein, that itself localizes to the primary cilium. VHL interacts with GSK-3 β and directly interacts with microtubules, supporting the stabilization and orientation that is crucial for maintaining the ciliary axoneme [75-77]. VHL is a tumor suppressor, polyubiquitin-ligase that normally targets various proteins including HIF1 α for degradation, and is commonly lost in kidney cancers. Ding et al. have shown that Nek8 is transcriptionally upregulated by loss of the ciliary stabilization factor pVHL in human renal cancer cell lines and speculated that Nek8 might play role in ciliary disassembly [47], although a connection to AURKA is not yet apparent. Dere and colleagues [78] identified a different mechanism for control of cilia in clear cell renal cell carcinoma (ccRCC) through transcriptional upregulation of the AURKA protein. This study found that in pVHL-deficient ccRCC cell lines, AURKA upregulation is driven by β -catenin-regulated transcription; while the increased HIF1 α activity in these cells partially inhibits β -catenin, the net effect is an AURKA increase. These studies revealed a complex dualistic role of pVHL in regulating primary cilia.

Nek8 and others: NEK8 (never in mitosis A-related kinase 8) has been recently suggested to have a function at cilia. In a study by Otto et al. several mutations in NEK8 gene were found in patients with nephronophthisis and some of them decreased its ciliary (L336F and H413Y) or centrosomal (H425Y) localization, although the Nek8 role on ciliogenesis and ciliary disassembly was not investigated [79]. It was also shown that defects in Nek8 ciliary localization might promote cystogenesis in kidneys [80] possibly through its influence on expression and localization of the polycystins PC1 and PC2 [81]. Zalli and colleagues have shown that Nek8 localizes at cilia and is degraded upon cells entering a quiescent state; however, although overall protein levels decrease, T162-phosphorylated (activated) Nek8 accumulates during cell cycle exit [82]. Grampa et al. found that mutations in Nek8 genes are associated with severe renal cystic dysplasia phenotype in human patients and in mouse models, in which Nek8 influences ciliogenesis and cell cycle, possibly through regulation of the nucleocytoplasmic shuttle of YAP, a main Hippo pathway effector. Interestingly, while Nek8 shRNA knockdown or overexpression of wild type Nek8 did not affect ciliogenesis, overexpression of cystic renal dysplasia-associated mutants of Nek8 (G580S and R602W) resulted in significant loss of cilia in cultured cells [48]. In patient fibroblasts with such mutants, there was increased YAP activation and nuclear localization. Interestingly, zebrafish embryos injected with either wild type or mutant Nek8 RNA exhibited a ciliopathy phenotype: an increased proportion of ventrally or dorsally curved body axis as well as laterality defects and pronephros abnormalities (cysts) and that phenotype was significantly restored by treatment with verteporfin, a YAP suppressor [83]. It will be of interest to determine if NEK8 functions to control AURKA activation.

Restriction of AURKA activity during ciliary assembly

After mitosis is complete, ciliogenesis occurs (Figure 2): this process has been thoroughly reviewed [84]. In brief introduction to the process of ciliogenesis to provide context for studies of AURKA activity control, immediately after mitosis, a group of distal appendage proteins (DAPs) that associate with the mother centriole, including Cep83, Cep89, Cep164, SCLT1 and FBF1, contribute to docking of post-mitotic centrioles to the membrane. The centriole differentiates to a basal body, anchoring primary cilium formation [85]. In cycling cells, the centriolar protein CP110 mechanistically forms a cap at the distal region of centrioles preventing axoneme extension. During ciliary assembly, a critical early event is the exclusion of CP110 from centrioles, which is required for axoneme formation [86]. While little is currently known about the exact mechanism of this molecular event or its upstream regulators, two kinases, Tau tubulin kinase 2 (TTBK2) [87] and microtubule affinity regulating kinase 4 (MARK4) [88] contribute to this process by phosphorylating one or more of components of the CP110/Cep97/Cep290/Kif24 ciliary assembly inhibitory complex. Interestingly, TTBK2 recruitment was shown to be dependent on PtdIns level that is being regulated by PIPK γ and INPP5E that, respectively, promote and inhibit TTBK2 recruitment to centriole, CP110 exclusion and thus ciliogenesis [89].

In parallel, Cep164 recruits Rabin8, a guanine nucleotide exchange factor, and the Rab8 GTPase to a pericentriolar vesicle [90], where the Rab11 GTPase stimulates the nucleotide exchange activity of Rabin8 [91], subsequently causing the activation and accumulation of Rab8 and primary ciliary vesicle formation [92, 93]. In parallel, PI3K Class II α (PI3K-C2 α) co-localizes with Rab11 at the ciliary base, and produces 3-phosphorylated phosphatidylinositol (PtdIns) that are required for Rab11 activation and subsequent Rab8 accumulation at ciliary base [94]. An additional component, the zinc-finger-containing protein Dzip1 supports Rab8 accumulation and activation at basal body [95]. Moreover, Dzip1 requires phosphorylation by GSK-3 β for activity in recruiting Rab8. Together, these proteins function to target specific proteins that are important for its function as a signaling hub through a ciliary transition zone and into the ciliary membrane.

Although AURKA levels are reduced after mitosis [96], a residual pool of the protein remains located at the ciliary basal body. Ciliary assembly requires this pool of AURKA remain inactive. Inactivity is assured in part by lack of signaling partners that promote AURKA activation. For example, NEDD9, a major AURKA activator, has been shown to accumulate at the centrosome during G2/M phase, but be expressed at a very low level in this intracellular compartment throughout the rest of cell cycle, except for a transient peak during specific induction of disassembly [49]. The AURKA activator Pifo also accumulates at centrosomes only at specific stages of the cell cycle – during early ciliary assembly, to prevent premature formation of cilium and during disassembly to facilitate it [51].

In part, inactivity of AURKA is achieved through the activity of proteins that negatively regulate AURKA. For example, Mergen et al. have shown that Inversin (NPHP2), a protein mutated in the ciliopathy nephronophthisis, can directly interact with both AURKA and its activator NEDD9, thus preventing AURKA activation at ciliary base [97]. An active process regulates the disappearance of trichoplein/TCHP specifically from the mother centriole but not the daughter centriole at the beginning of ciliogenesis; as the mother centriole is the

source of the nascent basal body of an assembling cilium, this suggested a potentially important regulatory role. Kasahara et al. demonstrated that this phenomenon occurs due to polyubiquitination of trichoplein by Cul3-RING E3 ligase (CRL3)–KCTD17 complex (CRL3^{KCTD17}) targeting it to proteasomal degradation [98]. Prior to primary cilium formation, trichoplein is targeted to degradation by CRL3^{KCTD17} ubiquitin-proteasome system releasing inhibitory effect of AURKA on primary cilium assembly [98].

The Hippo/YAP pathway is important for regulating contact inhibition and actin remodeling: in cultured cells, this process promotes ciliary assembly [99]. Interestingly, two major Hippo pathway components, the kinases MST1 and MST2, together with an activating scaffolding protein, SAV1, were shown to co-localize at ciliary base, and promote ciliogenesis. Kim and colleagues found that these MST kinases phosphorylate AURKA preferentially on serines of N-terminal fragment (although the exact sites were not identified), and that this phosphorylation disrupts AURKA-HDAC6 association and formation of ciliary disassembly complex. In parallel, MST1/2 proteins promote ciliary assembly through their association with NPHP proteins to regulating trafficking of various cargos through the ciliary transition zone, including Rab8a, Smo and RPGR [100].

AURKA, ciliopathies and cancer

Mutations in genes encoding proteins required for ciliogenesis have been identified as causal for a large number of genetic disorders that are classified as ciliopathies, with some of these directly connected to regulation of AURKA activity (Figure 3). Ciliopathies are typically hereditary syndromes with defects of primary cilia, and are associated with a wide range of overlapping symptoms. Several recent reviews elucidate the relationship between cilia and these pathologies [101-103]. One of the most studied ciliopathies is autosomal dominant polycystic kidney disease (ADPKD), which is the fourth leading cause of renal failure worldwide in adults and affects approximately 1 in 400 people [104, 105]. Autosomal recessive polycystic kidney disease (ARPKD) is the second common hereditary kidney disorder, more widespread among children [106, 107]. Birt-Hogg-Dube (BHD) syndrome is an autosomal dominant disorder where patients are predisposed to kidney cancer, lung and kidney cysts and benign skin tumors [108]. Less common ciliopathies are some of the oral-facial-digital (OFD) syndromes, such as Joubert syndrome, Meckel-Gruber syndrome and orofacioidigital syndrome type 1 [109-113]. Other ciliopathies include, but are not limited to retinal dystrophies, such as Leber's congenital amaurosis [114], Senior-Løken syndrome [115], Bardet-Biedl syndrome [116, 117], Alström syndrome [118] and other types of nephronophthisis [119]. Ciliary disorders of the skeleton are found in Jeune asphyxiating thoracic dystrophy [119, 120], Ellisvan Creveld syndrome, Sensen-Brenner syndrome (cranioectodermal dysplasia) and several others [121]. Some ciliary proteins contribute to regulation of metabolism and vascular development, with dysfunctions in components of the ciliary proteome leading to an increased risk of obesity [122-124] and predispose to the development of atherosclerotic plaques [125, 126].

Pathological activation of AURKA has been observed for some of the ciliopathies, with particular focus on studies in ADPKD, the most common and most studied ciliopathy REFs. The non-mitotic activation of AURKA was observed in pre-clinical models of ADPKD,

where it was shown to be regulated by $\text{Ca}^{2+}/\text{CaM}$ binding [59]. Further, misregulation of AURKA and its direct activator NEDD9 were shown to play important roles in promoting cystogenesis in a murine model of ADPKD [127]. In this study, the authors observed that in mice genetically null for NEDD9, a major AURKA activator, exhibited multiple abnormalities in cilia, including elongated cilia and multiciliated cells. Interestingly, when combined with conditional PKD1 knockout, a NEDD9 null genotype significantly exacerbated the cystic phenotype, although NEDD9 null mice did not generate cysts in the absence of a PKD1 driver lesion [127]. Similar findings also were observed after administration of AURKA inhibitors in PKD1 mutant mice, therefore suggesting caution in use of these agents in management of ADPKD and cancer therapies [127, 128]. In addition, mutations in INPP5E [65] cause the ciliopathies known as Joubert [129] and MORM (mental retardation, truncal obesity, retinal dystrophy and micropenis) syndromes [130]. Thereby, over the last decade multiple studies have revealed a direct link between additional non-mitotic AURKA functions including control of ciliary stability and calcium signaling and ciliopathies.

A growing number of studies and reviews have addressed the topic of possible connections between ciliary dynamics and cancer. Cilia are lost in many types of cancer, leading to the idea that they may contribute to cell division checkpoints (although they are retained in some tumor types, such as medulloblastomas, basal cell carcinomas and epithelial ovarian carcinomas which are often dependent on Hedgehog (HH) signaling. Thus, Egeberg and colleagues demonstrated, that ciliogenesis in ovarian tumors might be impaired due to overexpression of AURKA, leading to aberrant HH signaling [131]. In addition, some linkages have been identified between ciliogenesis and the cancer-relevant process of DNA damage response (DDR) regulation [132]. An extended discussion of this field is beyond the scope of this article, with several reviews recently published on this topic [133-135]. However, in specific relationship to control of AURKA, a well-known hallmark of clear cell renal carcinoma (ccRCC) is loss of the primary cilium, driven by loss of VHL gene and increased AURKA/HDAC6 activities [78]; while overexpression of the AURKA activator NEDD9 is common and promotes metastasis in many cancers [136], which may be linked in part to loss of cilia.

Conclusions

The data summarized above emphasize the multiple mechanisms for control of AURKA in non-mitotic cells, identified through study of ciliary dynamics. These include activation by Pifo, NEDD9, calmodulin, trichoplein, HIF1 α , Plk1, β -catenin, and Dvl2, and inhibition by NPHP2, PtdIns, MST1/2-SAV1. For many of these mechanisms, including regulation by Pifo, trichoplein, Nde1, CPAP, and the Nek2-Kif24 pathway, Nek8, their relevance to mitotic activation of AURKA is essentially unknown, and requires investigation. Some studies of AURKA small molecule inhibitors, including some under investigation as cancer therapeutics, have shown that interaction of AURKA with specific mitotic activation partners such as NEDD9 or TPX2 influence the activity of inhibitors [137, 138]. For more recently defined ciliary partners, whether these agents affect activity profile of inhibitors requires further study. It is also possible that drugs targeting AURKA might exhibit unanticipated adverse effects from cilia stabilization in both normal and malignant cells. Given the rapid

increase in studies demonstrating importance of the primary cilium in cell differentiation, proliferation, stem cell status, and other processes, it may be useful to include assessment of ciliary effects for drugs targeting AURKA and related partner proteins.

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Figure 1. Scheme of signaling pathways, regulating AURKA activity during ciliary disassembly
See description in text.

Figure 2. Scheme, representing role of AURKA in ciliogenesis
See description in text.

Figure 3. Ciliary defects in ciliopathies and cancer
Schematically represented summary of alterations in different genes and signaling pathways (*highlighted in italic*) affecting the primary cilium that lead to development of the diseases indicated. *AURKA* is highlighted in red, when there is published evidence of its involvement. Pathologies are clustered into three groups: ciliopathies (grouped by most common distinguished feature), cancer (with presence and loss of cilia reported), and other pathologies. Abbreviations: VHL – Von Hippel-Lindau, HH – Hedgehog signaling.