Cell Cycle–Dependent Ciliogenesis and Cancer

Olga V. Plotnikova,[1,2] Erica A. Golemis,[1] and Elena N. Pugacheva[3]

[1]Fox Chase Cancer Center, Philadelphia, Pennsylvania; [2]Department of Molecular Biology and Medical Biotechnology, Russian State Medical University, Moscow, Russia; and [3]Department of Biochemistry, Mary Babb Randolph Cancer Center; and West Virginia University, School of Medicine, Morgantown, West Virginia

Abstract

In mammals, most cell types have primary cilia, protruding structures involved in sensing mechanical and chemical signals from the extracellular environment that act as major communication hubs for signaling controlling cell differentiation and polarity. The list of clinical disorders associated with ciliary dysfunction has expanded from polycystic kidney disease to include many others. Transformed cells commonly lack cilia, but whether this lack is cause or consequence of transformation is not well understood. Here we discuss work addressing recently identified actions of the cancer-promoting proteins Aurora A and HEF1/NEDD9/CAS-L at cilia. Together with older studies, this work suggests that loss of cilia in cancer may contribute to the insensitivity of cancer cells to environmental repressive signals, based in part on derangement of cell cycle checkpoints governed by cilia and centrosomes. [Cancer Res 2008;68(7):2058–61]

Introduction

Immotile primary cilia are found on most epithelial and stromal cells in the mammalian body. The primary cilium is a centriole-based organelle that consists of (9+0) microtubule pairs located at the plasma membrane. The past decade has seen exponential growth in studies of the cilium, based on the recognition that numerous developmental defects and chronic diseases arise from aberrant formation or function of cilium (1). The idea of the cilium as "cellular cybernetic probe" (i.e., force and small molecule–sensing protrusion; ref. 2) capable of transducing environmental information from the extracellular matrix to the centrosome and suggesting a matrix-cilium-Golgi signaling continuum (2) was first proposed in the 1990s. This model is now supported by extensive data and informs studies of "ciliary diseases" such as polycystic kidney disease (PKD), Bardet-Biedl syndrome, and Kartagener's syndrome.

A correlation between cellular transformation and loss of a primary cilium has been noted for over a decade (3). It has been proposed that cancer cells lacking cilia reduce or alter their response to extracellular cues that regulate growth and differentiation. A number of recent studies have shown that a number of critical cell signaling and adhesion molecules are clustered on the cilium and require an intact cilium for normal function (Fig. 1*A*). These include components of the Sonic Hedgehog (Shh), planar cell polarity (PCP), platelet derived growth factor receptor α (PDGFRα), and von Hippel-Lindau and glycogen synthase kinase 3 β (VHL/GSK3β) pathways (4–6). For at least VHL/GSK3β, deregulated expression of the signaling protein has been found to influence the ability of cells to form cilia (7). Nevertheless, the significance of ciliary loss for influencing signaling in cancer development is largely unclear.

Overexpression of HEF1 is associated with metastatic progression of melanoma and lung cancer (8, 9), whereas Aurora A overexpression is a common oncogenic lesion in multiple cancer types (10). Overexpression of HEF1 and Aurora A promotes cytokinetic failure, a contributor to genomic instability; overexpression of HEF1 also induces tumor cell migration and invasion. As discussed below, the recent definition of a HEF1-Aurora A-HDAC6 signaling axis governing the resorption of cilia in addition to the previously defined roles for these proteins suggests a novel molecular mechanism to explain at least some cancer-associated ciliary loss. We propose that enhanced intracellular levels of HEF1 and Aurora A may also interfere with a normal cellular interconversion between cilia and centrosome that helps time cell cycle by staging critical signaling complexes that govern emergence from quiescence, entry to S phase, and initiation of M phase. Through these actions, HEF1 and Aurora A can provide multiple stimuli to cancer aggressiveness.

Cell Cycle–Dependent Ciliogenesis

The transition between centrosome and cilium and the regulation of cell cycle control are closely linked in normal dividing cells. Centrosomes, which are composed of two barrel-shaped centrioles surrounded by pericentriolar material, organize the cytoplasmic microtubule network during interphase and the bipolar spindle during mitosis. In postmitotic cells, the centrosome migrates to the cell surface, and one of the centrioles differentiates into a basal body that nucleates microtubules to form a cilium (11). In normal proliferating cells, the presence of a cilium on the cell surface is transient: most commonly observed in G_1 and usually resorbed before or during G_2 phase. In asynchronous populations, typically 10% to 25% of cells have observable cilia (12). As cultured cells become quiescent, an increasing proportion of the population is ciliated, attaining >90% in some cell types. This quiescent ciliated state parallels the natural state of normal cells organized in tissues *in vivo* and persists until environmental changes trigger cell cycle re-entry.

An increasing number of factors controlling the formation or loss of cilia in normal cell cycle are being found to influence cell cycle. Regarding ciliary formation, removal of a number of intraflagellar transport (IFT) proteins causes severe ciliary assembly defects or complete loss of cilium (13). Some of the IFT proteins, long thought to solely be relevant to protein trafficking within cilia, have recently been found to function in cell cycle control. For example, reduced levels of IFT27 results in cell growth inhibition and incomplete or asymmetrical cytokinesis (14). Overexpression of IFT88 prevents G_1-S transition, whereas depletion of IFT88 causes cilium disappearance and promotes cell cycle

Requests for reprints: Elena N. Pugacheva, Department of Biochemistry, Mary Babb Randolph Cancer Center, West Virginia University, School of Medicine, Morgantown, WV 26506-9300. Phone: 304-293-5295; Fax: 304-293-4667; E-mail: epugacheva@hsc.wvu.edu.

doi:10.1158/0008-5472.CAN-07-5838

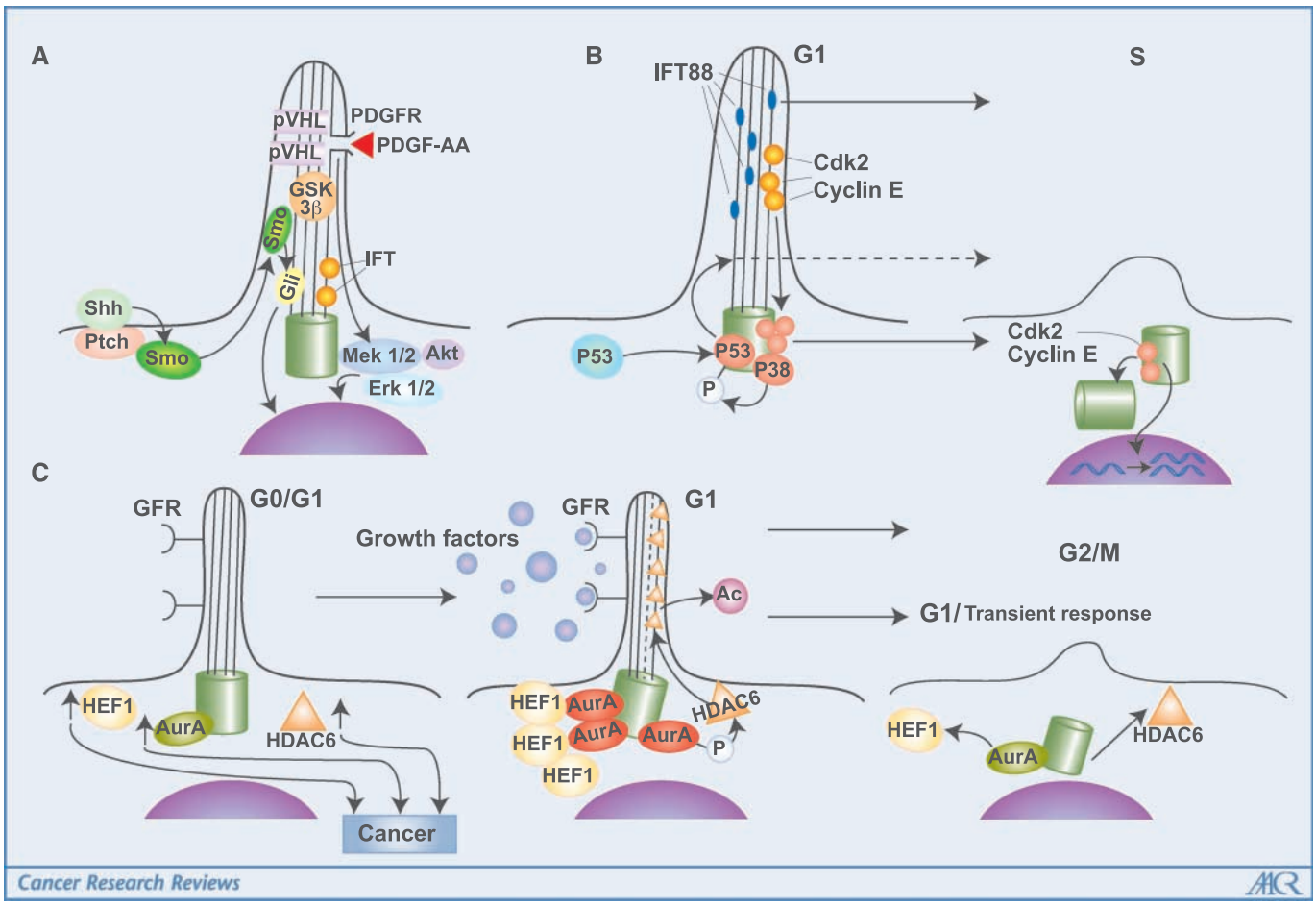

Figure 1. *A,* the cilium as an environmental rheostat: coordination of cancer-related signaling molecules. PDGF-AA binding to the PDGFRαα receptor located at the ciliary membrane induces phosphorylation and activation of the Akt and Mek1/2-Erk1/2 signaling pathways. Shh binding to Patched (*Ptch*) relieves inhibition of the Smoothened protein (Smo) and initiates a signaling cascade that result in activation of the Gli family of zinc-finger transcription factors. Localization of the Smo receptor to primary cilia is important for this signaling cascade. Aberrant Shh signaling was recently observed in a variety of epithelial cancers, and it has been shown that the Shh target Gli1 regulates expression of *Snail*, a gene important for epithelial-mesenchymal transition. pVHL and GSK-3β also localize to primary cilia and act together to maintain the primary cilium: their combined inactivation leads to loss of cilia and correlates with renal tumor progression. *B,* centrosome serves as the signaling molecule hub licensing cell cycle progression. As cells emerge from quiescence and resorb cilia, the CDK2-cyclin E complex progressively associates with the centrosome and triggers the G_1-S transition. Centrosome duplication and DNA synthesis are tightly connected through cyclin E/Cdk2 activation. Overexpression of IFT88 prevents G_1-S transition, whereas depletion of IFT88 induces cell cycle progression. The cell cycle checkpoint regulator p53 evaluates centrosome integrity in cell cycle. p53 accumulation at the centrosome is preceded by p38-dependent phosphorylation and is important for execution of G_1-S arrest. Deletion or mutations in p53 gene were found in >50% of all human cancers: consequences, if any, for ciliary integrity are not known. *C,* model for HEF1-Aurora A-HDAC6 signaling at cilia. Extracellular factors bind to growth factor receptors to induce HEF1. HEF1 binds to Aurora A kinase, promoting its activation. Aurora A in turn phosphorylates and activates cilia-associated HDAC6. Moving along the ciliary axomene, HDAC6 deacetylates substrates, causing ciliary resorption. These events occur as an early transient response of G_0-G_1 cells to serum treatment as well as at G_2-M transition in response to intrinsic signaling cues.

progression (15). As the ciliary membrane is strongly enriched with growth factor, chemosensing, and mechanosensing receptors (13, 16), absence of cilia may partly influence cell cycle progression by limiting the ability of cells to respond to external cues.

As an alternative explanation, some evidence suggests that misregulation of the basal body–centrosome transition may trigger cell cycle checkpoints. A centrosome is becoming well-established as an organization center for signaling proteins that drive S-phase progression (17), emphasized by studies demonstrating loss of cell cycle progression after centrosome ablation (18). An early study suggested that at least in some model systems, centrioles encompassed within a ciliary basal body do not seem to be competent to organize the factors necessary to progress through S phase (19). Rather, the basal body "differentiates" back into a centrosome before performing these functions, which include spatially coordinating the activation of the Cdk2/cyclin E kinase complex (20), necessary both for centrosome duplication and DNA synthesis (21). However, as mice lacking Cdk2 are phenotypically normal, with only a delay in S-phase entry by $Cdk2^{-/-}$ cells (22), and as some cell types can progress through S phase although their centrosomes are entirely ablated (18), activation of centrosomal Cdk2/cyclin E is not essential in all cell types, potentially due to redundant pathways. It has also been proposed that overexpressed IFT88 induces cell cycle arrest at least in part by activating the Rb growth suppressor (15). Interestingly, a recent detailed analysis has shown that siRNA depletion of a panel of proteins required for core centrosomal structural integrity both induces G_1 arrest by triggering a p53/p38-dependent checkpoint and inhibits formation of primary cilia (Fig. 1*B*; ref. 23). The relative priority of these events in the arrest process requires further study.

Although some cell types transition through S phase without resorbing cilia, almost no examples exist where ciliated cells enter

mitosis, suggesting a second restriction point of intact cilia on cell cycle (12). In a number of systems, a centrosome is not necessary for spindle formation (24) or mitotic entry, although an important centrosomal role in timing mitotic entry based on spatially coordination activation of the Aurora A kinase to allow nuclear envelope breakdown has recently been described (25). It is possible that a mother centriole sequestered in a basal body has an actively inhibitory role on mitotic entry, although no experiments to date have directly tested this idea. Suggestively, a cilia-associated member of the NIMA family of cell cycle kinases, Fa2p, is necessary for both cell division–regulated ciliary resorption and cell cycle progression from G_2 to M phase (16, 26).

Clearly, although a growing body of evidence links cilia function and cell cycle in normal cells, none of the preceding studies have closely implicated the ciliary resorption/protrusion cycle with changes relevant to cancer. In the context of the above discussion, the typical absence of cilia from transformed cells suggest several possible interpretations for a cancer-promoting role of the altered state: (*a*) it may be beneficial to some classes of tumor cells to limit responsiveness to external cues that promote differentiation over cycling; and (*b*) the continual presence of centrioles in a centrosomal rather than ciliary context may remove cilia-initiated cell cycle restrictions. Such restriction cannot be universal for mammalian cancer; some cell types, notably hematopoietic cells, are nonciliated. However, particularly for cancers associated with aberrant signaling involving PDGF, Wnt, or Hedgehog signaling, such a ciliary contribution may be key. To better assess such a model, it is important to understand the factors controlling ciliary loss in cancer.

HEF1-Dependent Activation of Oncogenic Aurora A Kinase Induces Ciliary Disassembly

Aurora A is a centrosomally localized cell cycle regulatory serine/threonine kinase that activates the cyclin B1-Cdk1 mitotic kinase and coordinates formation of a bipolar spindle and nuclear envelope breakdown in M phase (25). Aurora A is overexpressed due to gene amplification in many types of human cancer (10). In both naturally occurring tumors and with ectopic overexpression systems, increased Aurora A correlates with induction of aneuploidy, centrosomal anomalies, tumor invasiveness, and poor prognosis. It has recently been proposed that increased Aurora A expression is the major contributing factor to the elevated instability and aneuploidy of p53 null tumors (27).

HEF1 has recently emerged as a prometastatic factor. Elevated expression of HEF1 marks melanomas, a subset of lung cancers, and other cancers, and for melanomas and glioblastomas, HEF1 clearly drives invasive behavior (9, 28). A significant amount of work over the past 12 years has elucidated complex HEF1 roles at focal adhesions in regulation of migration, apoptosis, and chemokine response (29, 30), processes relevant to metastasis. However, in 2005, a centrosomal pool of HEF1 was found to bind and help activate the Aurora A kinase at mitotic entry (31), linking function of the two proteins. Increased HEF1 expression produced similar defects in cytokinesis and centrosome amplification as increased levels of Aurora A, suggesting a potential role for HEF1 in promoting aneuploidy in metastatic tumors.

Providing a critical link to the idea of a role for HEF1 and Aurora A in the ciliary cycle, in 2004, Snell and coworkers (32) had determined that CALK, a *Chlamydomonas* protein that is distantly related to Aurora A, governs resorption of the flagellum, an organelle similar to the mammalian cilium. Intriguingly, in *Chlamydomonas*, CALK is not solely a mitotic kinase but functions throughout cell cycle in response to a variety of cues inducing loss of flagella. Pursuing these hints, we subsequently showed HEF1-dependent activation of Aurora A upon induction of ciliary resorption by growth factor treatment of quiescent cells (Fig. 1*C*; ref. 33). We also established the requirement for Aurora A kinase for cilium disassembly not only during mitosis but also in G_1 cells emerging from quiescence: the first time a nonmitotic activity for Aurora A had been identified in mammalian cells and paralleling activity in *Chlamydomonas*. Furthermore, microinjection of Aurora A into cells resulted in almost instantaneous cilium disappearance, leading to the conclusion that active Aurora A is necessary and sufficient to induce cilium resorption. Interestingly, a recent study of male infertility has established a mutation leading to loss of expression of the Aurora A–related kinase; Aurora C caused production of large-headed, multiflagellar spermatozoa (34), indirectly suggesting a related activity.

Moving downstream, Aurora A specifically phosphorylates the tubulin deacetylase HDAC6, dramatically increasing in HDAC6 deacetylase activity. Inhibition of HDAC6 activity by the small molecule inhibitor tubacin or gene silencing of HDAC6 by siRNA, in each case, resulted in cilium stabilization, thus establishing HDAC6 as one important downstream targets of Aurora A kinase involved in cilium destabilization. HDAC6 activity is most simply explained through action regulating the ciliary axoneme, removing stabilizing acetylation marks on axonemal α-tubulin. However, some earlier studies in Chlamydomonas have argued that changes in the acetylation status of α-tubulin do not influence flagellar resorption (35); hence, either *Chlamydomonas* and mammals may be different in this regard or HDAC6 may target additional or alternative substrates in this process. At least indirectly, one such target may be Aurora A itself; a very recent study has shown that inhibition of HDAC6 caused destabilization of Aurora A kinase via disruption of an HSP90-Aurora A complex (36). It is likely that additional Aurora A and HDAC6 targets will be identified, with IFT proteins likely candidates for activity-altering modification.

Tipping the Cilium-Centrosome Balance as a Procancer Stimulus?

As reviewed above, a growing body of data suggests that structural and signaling integrity of the cilium are important for regulation of a normal cell cycle. These data indicate timed resorption and reassembly of cilia are dynamic processes closely linked to execution of cell developmental programs, and suggest the following speculative model (Fig. 1*C*): in quiescent, G_0-G_1 cells of lineages that are typically ciliated, receptors for growth factors, or other differentiation control signals (eg., PDGFR/Ptch-Smo) are displayed on the ciliary membrane. Upon stimulation of these receptors, a signal is transported down to the command center at the basal body. This induces "firing" of the Aurora A kinase, promoting local activation of HDAC6, transporting it along axoneme to destabilize microtubules, and/or influence the activity of IFTs. Again speculatively, in normal cells organized in tissues (not actively proliferating), one purpose of transient Aurora A–HDAC6 activation after receipt of transient growth factor stimulation may be to control ciliary length. Fine control of ciliary length may serve as a rheostat, increasing or decreasing the ability of cells to respond to growth factors by limiting area for display of cilia-associated receptors. In contrast, strong activation of Aurora

A and HDAC6 is sufficient to induce full ciliary resorption and remove basal body–dependent restrictions on cell cycle progression, with the basal body centriole differentiating to a centrosome competent to fully coordinate S- and M-phase entry. Finally, abnormal overexpression and/or activation of key components of this resorption signaling machinery such as HEF1 and Aurora A enforces ciliary disassembly under all conditions, distorting the responsiveness of cells to a subset of external growth regulatory cues (37) and inducing excessive cell proliferation and migration.

The relations between control of ciliary integrity, ciliary signaling, and cancer clearly require further study. Provocatively, besides the role of cilia in coordinating PDGFα and Hedgehog signaling, disruption of the ciliary basal body has very recently been shown to also cause defective signaling in the Wnt pathway: Wnt signaling is commonly perturbed in many cancers. In lung cancer metastases dependent on loss of the tumor suppressor LKB1, HEF1 is strongly up-regulated (8); interestingly, LKB1 also regulates Wnt signaling (38), and mutational inactivation of LKB1 in development phenocopies loss of VHL, another regulator of cilia (39). A common role of LKB1, Wnt, and VHL is in the regulation of PCP. PCP control is extremely important in known ciliary diseases, such as PKD, with aberrant mitotic orientation found to contribute to renal tubular enlargement and cyst formation (40). It seems likely that LKB1 might be an upstream regulator of ciliary resorption through control of HEF1, Aurora A, and HDAC6. Whether HEF1, Aurora A, and HDAC6 in turn influence PCP via actions at centrosomes and cilia is an as-yet uninvestigated but intriguing possibility.

Based on the well-established role of Aurora A and HDAC6 in regulating mitosis, several inhibitors targeting these proteins have been developed and have entered phase I clinical trials for advanced cancer treatment. The new ciliary functions of Aurora A kinase and HDAC6 suggest the necessity of a more complicated interpretation of the output of Aurora A- and HDAC-directed therapeutics. Further research directed at understanding the relationship between the cilium, cell cycle, and cilia-mediated signaling is likely to reveal new insights into the pathogenesis of many types of cancer and nominate additional cilia-associated proteins as new targets for cancer therapeutics.

Acknowledgments

Received 10/11/2007; revised 12/6/2007; accepted 12/14/2007.

Grant support: NIH RO1 CA63366 and RO1 CA113342, a Department of Defense OCRF IDEA Award (E.A. Golemis), Pennsylvania Tobacco Settlement Funds (to E.A. Golemis), by an Appropriation from the Commonwealth of Pennsylvania, by NIH core grant CA06927 (to Fox Chase Cancer Center), and West Virginia University, Robert C. Byrd Health Sciences Center's Strategic Research Plan (E.N. Pugacheva). We apologize to our colleagues whose outstanding publications we have not been able to directly cite due to space constraints.

References

1. Eggenschwiler JT, Anderson KV. Cilia and developmental signaling. Annu Rev Cell Dev Biol 2007;23:345–73.
2. Poole CA, Jensen CG, Snyder JA, Gray CG, Hermanutz VL, Wheatley DN. Confocal analysis of primary cilia structure and colocalization with the Golgi apparatus in chondrocytes and aortic smooth muscle cells. Cell Biol Int 1997;21:483–94.
3. Wheatley DN. Primary cilia in normal and pathological tissues. Pathobiology 1995;63:222–38.
4. Ciruna B, Jenny A, Lee D, Mlodzik M, Schier AF. Planar cell polarity signalling couples cell division and morphogenesis during neurulation. Nature 2006;439:220–4.
5. Corbit KC, Aanstad P, Singla V, Norman AR, Stainier DY, Reiter JF. Vertebrate Smoothened functions at the primary cilium. Nature 2005;437:1018–21.
6. Schneider L, Clement CA, Teilmann SC, et al. PDGFRαα signaling is regulated through the primary cilium in fibroblasts. Curr Biol 2005;15:1861–6.
7. Thoma CR, Frew IJ, Hoerner CR, Montani M, Moch H, Krek W. pVHL and GSK3β are components of a primary cilium-maintenance signalling network. Nat Cell Biol 2007;9:588–95.
8. Ji H, Ramsey MR, Hayes DN, et al. LKB1 modulates lung cancer differentiation and metastasis. Nature 2007;448:807–10.
9. Kim M, Gans JD, Nogueira C, et al. Comparative oncogenomics identifies NEDD9 as a melanoma metastasis gene. Cell 2006;125:1269–81.
10. Katayama H, Brinkley WR, Sen S. The Aurora kinases: role in cell transformation and tumorigenesis. Cancer Metastasis Rev 2003;22:451–64.
11. Dawe HR, Farr H, Gull K. Centriole/basal body morphogenesis and migration during ciliogenesis in animal cells. J Cell Sci 2007;120:7–15.
12. Rieder CL, Jensen CG, Jensen LC. The resorption of primary cilia during mitosis in a vertebrate (PtK1) cell line. J Ultrastruct Res 1979;68:173–85.
13. Yoder BK. More than just the postal service: novel roles for IFT proteins in signal transduction. Dev Cell 2006;10:541–2.
14. Qin H, Wang Z, Diener D, Rosenbaum J. Intraflagellar transport protein 27 is a small G protein involved in cell-cycle control. Curr Biol 2007;17:193–202.
15. Robert A, Margall-Ducos G, Guidotti JE, et al. The intraflagellar transport component IFT88/polaris is a centrosomal protein regulating G_1-S transition in non-ciliated cells. J Cell Sci 2007;120:628–37.
16. Quarmby LM, Parker JD. Cilia and the cell cycle? J Cell Biol 2005;169:707–10.
17. Sluder G. Two-way traffic: centrosomes and the cell cycle. Nat Rev Mol Cell Biol 2005;6:743–8.
18. Uetake Y, Loncarek J, Nordberg JJ, et al. Cell cycle progression and *de novo* centriole assembly after centrosomal removal in untransformed human cells. J Cell Biol 2007;176:173–82.
19. Tucker RW, Pardee AB, Fujiwara K. Centriole ciliation is related to quiescence and DNA synthesis in 3T3 cells. Cell 1979;17:527–35.
20. Tsou MF, Stearns T. Mechanism limiting centrosome duplication to once per cell cycle. Nature 2006;442:947–51.
21. Nigg EA. Centrosome duplication: of rules and licenses. Trends Cell Biol 2007;17:215–21.
22. Berthet C, Aleem E, Coppola V, Tessarollo L, Kaldis P. Cdk2 knockout mice are viable. Curr Biol 2003;13:1775–85.
23. Mikule K, Delaval B, Kaldis P, Jurcyzk A, Hergert P, Doxsey S. Loss of centrosome integrity induces p38-53-p21-dependent G_1-S arrest. Nat Cell Biol 2007;9:160–70.
24. Basto R, Lau J, Vinogradova T, et al. Flies without centrioles. Cell 2006;125:1375–86.
25. Portier N, Audhya A, Maddox PS, et al. A microtubule-independent role for centrosomes and aurora a in nuclear envelope breakdown. Dev Cell 2007;12:515–29.
26. Mahjoub MR, Qasim Rasi M, Quarmby LM. A NIMA-related kinase, Fa2p, localizes to a novel site in the proximal cilia of Chlamydomonas and mouse kidney cells. Mol Biol Cell 2004;15:5172–86.
27. Mao JH, Wu D, Perez-Losada J, et al. Crosstalk between Aurora-A and p53: frequent deletion or down-regulation of Aurora-A in tumors from p53 null mice. Cancer Cell 2007;11:161–73.
28. Natarajan M, Stewart JE, Golemis EA, et al. HEF1 is a necessary and specific downstream effector of FAK that promotes the migration of glioblastoma cells. Oncogene 2006;25:1721–32.
29. O'Neill GM, Fashena SJ, Golemis EA. Integrin signalling: a new Cas(t) of characters enters the stage. Trends Cell Biol 2000;10:111–9.
30. Singh M, Cowell L, Seo S, O'Neill G, Golemis E. Molecular basis for HEF1/NEDD9/Cas-L action as a multifunctional co-ordinator of invasion, apoptosis and cell cycle. Cell Biochem Biophys 2007;48:54–72.
31. Pugacheva EN, Golemis EA. The focal adhesion scaffolding protein HEF1 regulates activation of the Aurora-A and Nek2 kinases at the centrosome. Nat Cell Biol 2005;7:937–46.
32. Pan J, Wang Q, Snell WJ. An aurora kinase is essential for flagellar disassembly in Chlamydomonas. Dev Cell 2004;6:445–51.
33. Pugacheva EN, Jablonski SA, Hartman TR, Henske EP, Golemis EA. HEF1-dependent Aurora A activation induces disassembly of the primary cilium. Cell 2007;129:1351–63.
34. Dieterich K, Soto Rifo R, Faure AK, et al. Homozygous mutation of AURKC yields large-headed polyploid spermatozoa and causes male infertility. Nat Genet 2007;39:661–5.
35. Kozminski KG, Diener DR, Rosenbaum JL. High level expression of nonacetylatable α-tubulin in Chlamydomonas reinhardtii. Cell Motil Cytoskeleton 1993;25:158–70.
36. Park JH, Jong HS, Kim SG, et al. Inhibitors of histone deacetylases induce tumor-selective cytotoxicity through modulating Aurora-A kinase. J Mol Med 2008;86(1):117–28. Epub 2007 Sep 13.
37. Huangfu D, Anderson KV. Cilia and Hedgehog responsiveness in the mouse. Proc Natl Acad Sci U S A 2005;102:11325–30.
38. Ossipova O, Bardeesy N, DePinho RA, Green JB. LKB1 (XEEK1) regulates Wnt signalling in vertebrate development. Nat Cell Biol 2003;5:889–94.
39. Ylikorkala A, Rossi DJ, Korsisaari N, et al. Vascular abnormalities and deregulation of VEGF in Lkb1-deficient mice. Science New York NY 2001;293:1323–6.
40. Fischer E, Legue E, Doyen A, et al. Defective planar cell polarity in polycystic kidney disease. Nat Genet 2006;38:21–3.