ELSEVIER

Cell cycle kinases in cancer

Marcos Malumbres[1] and Mariano Barbacid[2]

Cell division in mammalian cells is driven by protein kinases that regulate progression through the various phases of the cell cycle. Cyclin-dependent kinases (Cdks) regulate cell cycle commitment, DNA synthesis and the onset of mitosis. Kinases of the Aurora, Polo and Nek families participate in the centrosome cycle and modulate spindle function. Additional kinases such as Bub1, BubR1 and Mps1 regulate the spindle assembly checkpoint. It has been well established that misregulation of Cdks is one of the most frequent alterations in human cancer. Recent evidence indicates that mutations involving mitotic kinases are also linked to tumor development. These findings suggest novel strategies to use cell cycle kinases as targets for therapeutic intervention.

Addresses
[1] Cell Division and Cancer, Molecular Oncology Programme, Centro Nacional de Investigaciones Oncológicas (CNIO), 28029 Madrid, Spain
[2] Experimental Oncology Groups, Molecular Oncology Programme, Centro Nacional de Investigaciones Oncológicas (CNIO), 28029 Madrid, Spain

Corresponding author: Barbacid, Mariano (barbacid@cnio.es)

Current Opinion in Genetics & Development 2007, **17**:60–65

This review comes from a themed issue on
Genetic and cellular mechanisms of oncogenesis
Edited by Sara A Courtneidge and Benjamin G Neel

0959-437X/$ – see front matter

DOI 10.1016/j.gde.2006.12.008

Introduction

To date, about 300 genes have been found to be mutated in at least one type of human cancer [1]. Many additional genes are likely to participate in tumor development by mechanisms that involve changes in expression levels (e.g. epigenetic silencing, transcriptional activation and microRNAs) or in their pattern of expression (e.g. ectopic expression and developmental misregulation). Unfortunately, only a limited number of 'cancer genes' encode drugable targets — targets for which suitable drugs can be generated. Among them, protein and lipid kinases provide some of the most suitable targets for small molecule inhibitors. The success of the breakpoint cluster region–Abelson (BCR–ABL) kinase inhibitor imanitib (known as Gleevec®) in the treatment of chronic myelogenous leukemia has provided the necessary 'proof of principle' for the use of kinase inhibitors in a clinical setting. Although kinases are implicated in most 'cancer pathways', the cell cycle has attracted special attention, given the direct relevance of cell proliferation to tumor development [2,3]. Accumulated evidence indicates that misregulation of cell cycle kinases might result in at least two cancer-associated defects: unscheduled proliferation and aberrant cell division leading to genomic instability. Here, we review recent findings on the involvement of several cell cycle kinases in these cancer pathways, along with current efforts aimed at designing novel therapeutic strategies to combat human cancer.

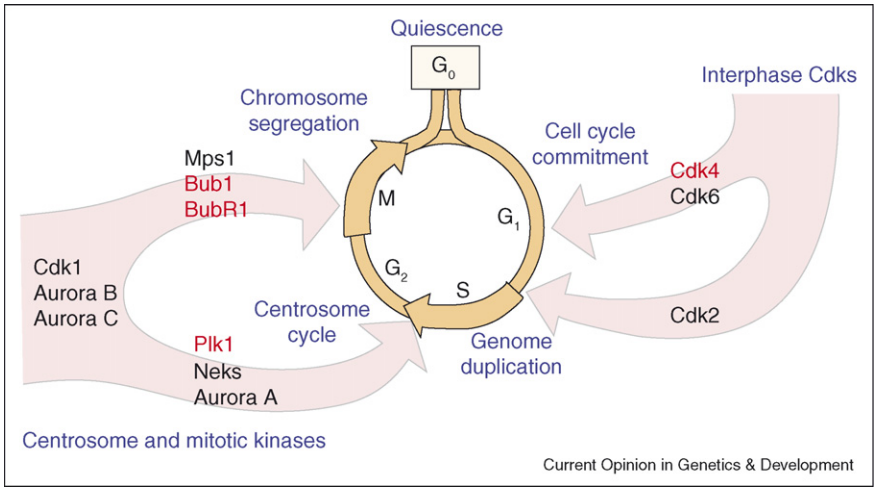
Cyclin-dependent kinases: controlling unscheduled proliferation of cancer cells

Cyclin-dependent kinases (Cdks) are heterodimeric protein kinases composed of a catalytic subunit known as Cdk and a regulatory subunit known as Cyclin. The mammalian genome has twelve loci encoding Cdks, although only five of them, *Cdk1*, *Cdk2*, *Cdk3*, *Cdk4* and *Cdk6* have been directly implicated in driving the cell cycle (Figure 1) [4,5]. Whereas Cdk1 is generally considered to be a mitotic kinase (see below), the other Cdks are believed to play a role in the early phases of cell division (interphase) [4]. To date, only *Cdk4* has been found mutated in human cancer, albeit only in a few cases of hereditary melanoma. In addition, *Cdk6* overexpression has also been documented in lymphomas, leukemias and melanomas as a consequence of chromosomal translocations [4]. However, a significant fraction of human cancers carry mutations that result in misregulation of Cdk activity. They include overexpression of their cognate Cyclins and inactivation of Cdk inhibitors, including members of the INK4 (Inhibitor of kinase 4) and Cip/Kip families. This information has been previously reviewed [2,4] and will not be further discussed here.

Cdks as targets for therapeutic intervention

The drugable nature of kinases in general, along with the frequent misregulation of Cdk activity in human cancer has led to intensive efforts to develop selective Cdk inhibitors. To date, none of them have successfully completed the early phases of clinical development, mainly due to toxic effects [3]. Yet recent genetic evidence suggests that at least some Cdks might be suitable targets for therapeutic intervention, at least in those tumors overexpressing D-type Cyclins. Ablation of Cyclin D1, one of the activating partners of Cdk4 and Cdk6, conferred resistance to mammary carcinogenesis induced by certain oncogenes such as Ras and ErbB2 [6]. Given that Cyclin D1 is essential for proper mammary gland development, the resistance of these mice to breast tumors could be due to a developmental defect rather than a lack of Cdk4 and Cdk6 activity. More recently, however, it has been shown that mice expressing a Cyclin

Figure 1



Main cell cycle kinases and their involvement in cell cycle progression. Kinases mutated in human cancer are shown in red.

D1 mutant that binds, but does not activate, Cdk4 or Cdk6 are resistant to breast cancer initiated by ErbB2, in spite of having normal mammary gland development (Table 1) [7••]. Similarly, *Cdk4* null mice, which display minor defects in normal mammary gland development, are also resistant to ErbB2-driven breast tumors (Table 1) [8••,9••]. These results strongly support the concept that Cdk4 and/or Cdk6 kinases might be relevant targets for therapeutic intervention, at least for breast cancers overexpressing *HER2*, the human homologue of *ErbB2* [10]. Another common defect observed in human tumors is loss or severe reduction in p27Kip1 protein levels, a defect linked to poor tumor prognosis [2]. p27Kip1 is a member of the Cip/Kip family of cell cycle inhibitors assumed to block cell division by directly interacting with Cdk2–CyclinE and Cdk2–CyclinA complexes, an interaction that results in inhibition of their kinase activity. Thus, it has been widely assumed that blocking Cdk2 activity with selective inhibitors should provide a therapeutic benefit. Unfortunately, ablation of Cdk2 in *p27Kip1* knockout mice has no effect on either tumor incidence or latency (Table 1) [11•,12•], thus raising doubts about the efficacy of this potential therapeutic strategy.

Mitotic kinases: protecting cells from chromosome aberrations

Aneuploidy is a landmark of many human cancers [13]. Recent evidence indicates that proper chromosome segregation is tightly controlled by mitotic kinases such as Cdk1 (also known as Cell division cycle 2 [Cdc2]), Polo-like kinases (Plks), and Aurora and Nek kinases (Figure 1). These enzymes are involved in regulating the centrosome cycle and formation of the mitotic spindle. Once the spindle is formed, the proper bipolar attachment of sister chromatides is mediated by the spindle assembly checkpoint (SAC), a signaling pathway that ensures proper connections between kinetochores and spindle microtubules [13]. Alterations in this pathway can result in either mitotic catastrophe that triggers apoptosis or exit from mitosis with aberrant chromosomal distribution. The SAC is controlled by the Bub kinases, Bub1 and BubR1, Aurora B and the kinetochore kinase Mps1 (Monopolar spindle 1), a dual Serine/Threonine and Tyrosine kinase also known as Ttk. Recent biochemical and genetic data have shed light on the role of these protein kinases in cell cycle regulation as well as on their implication in human cancer (Table 2).

Table 1

Genetic approaches for preclinical evaluation of Cdks as therapeutic targets.

Kinase	Model	Phenotype	References
Cdk2	*Cdk2*$^{-/-}$;*p27Kip1*$^{-/-}$	These mice develop tumors with incidence and latency similar to *p27Kip1*$^{-/-}$ mice, suggesting that tumor suppressor function of p27Kip1 is independent of Cdk2	[11•,12•]
Cdk4	*Cdk4*$^{-/-}$;*MMTV–ErbB2*	Resistant to ErbB2-induced breast tumors	[8••,9••]
Cdk4/6	*Cyclin D1*$^{K112E/K112E}$;*MMTV–ErbB2*	Resistant to ErbB2-induced breast tumors	[7••]

Abbreviations: MMTV, mouse mammary tumour virus.

Table 2

Representative mouse tumor models of cell cycle kinases.

Kinase	Model	Phenotype	References
Cdk4	*Cdk4*$^{R24C/R24C}$	Mice expressing an endogenous INK4-insensitive Cdk4^{R24C} mutant protein develop a variety of tumor types with complete penetrance after long latency (12–28 months).	[45]
Cdk4	*Cdk4*$^{R24C/R24C}$ +DMBA	Mice develop invasive melanoma with properties (S100 expression and progressive loss of melanin) similar to those observed in human patients.	[46]
Cdk4	*Cdk4*$^{R24C/R24C}$; *p27Kip1*$^{-/-}$	Mice develop aggressive pituitary tumors with short latency (8–10 weeks).	[47]
Aurora A	*Aurora A* transgenics	Mice develop mammary gland hyperplasia with significant presence of binucleated cells.	[48]
Plk4	*Plk4*$^{+/-}$	Mice develop lung and liver tumors. Wild type allele is retained in tumors.	[28••]
BubR1	*Bub1B*$^{+/-}$	Mice display progeroid syndrome and infertility. They also show increased susceptibility to lung and intestinal neoplasias.	[36••,37••]
BubR1	*Bub1B*$^{+/-}$;*Apc* ^{Min}/+	Lower levels of BubR1 increases incidence and aggressiveness of tumors in *Apc*$^{Min/+}$ mutant mice.	[49]

Abbreviations: Apc, adenomatous polyposis coli.

Cdk1

Cdk1, when bound to Cyclin A or Cyclin B, promotes centrosome duplication, spindle assembly and chromosome condensation, among other functions [4]. Specific inhibition of Cdk1 before mitosis results in G_2 arrest, whereas inhibition during mitosis provokes a quick exit from mitosis without cytokinesis [14]. Although the role of this kinase during the cell cycle has been extensively analyzed, its implication in tumor development and, hence, as a target for cancer therapy has not been properly evaluated. Yet it is currently assumed that tampering with Cdk1 will result in intolerable toxic effects.

Aurora kinases

Aurora kinases, including Aurora A, B and C, are key regulators of mammalian cell division. Aurora A localizes to centrosomes and spindle poles during mitosis, and its inhibition results in centrosome separation defects [15]. Aurora B, by contrast, is a chromosomal passenger implicated in SAC. The localization of this kinase at the mitotic apparatus varies depending upon the stage of the cell cycle. Aurora C is structurally and functionally related to Aurora B, although its expression is restricted to certain cell lineages [15]. Aurora kinases are essential to ensure error-free cell division, and their overexpression appears to be intimately linked to centrosome amplification, malignant transformation and resistance to microtubule poisons [15,16]. Aurora A phosphorylates tumor suppressors such as p53, thereby modulating their activities [16]. More recently, Aurora A and B have also been shown to cooperate with Ras-mediated cell transformation [17–19]. Inhibition of Aurora A by RNA interference or by small molecule inhibitors results in a monopolar phenotype that prevents chromosome segregation [15,20•]. By contrast, inhibition of Aurora B provokes exit from mitosis without cytokinesis, and loss of viability, suggesting distinct therapeutic values for these two kinases [20•]. Recently, Aurora kinases have been implicated in a gene expression signature for chromosomal instability [21••]. Among the 70 genes represented in this signature, overexpression of TPX2 (Targeting protein for *Xenopus* kinesin-like protein 2), an activator of Aurora A, showed the highest correlation to chromosomal instability. Overexpression of Aurora A is also included in this signature along with other genes encoding mitotic kinases such as Aurora B, Cdk1, Nek2 and Mps1 (see below). Overexpression of these genes correlates with poor clinical outcome in tumors with chromosomal instability [21••]. These observations, taken together, have led to intense efforts to develop Aurora kinase inhibitors, some of which are currently under clinical evaluation [16].

Plk1 and other Polo-like kinases

The best characterized member of the mammalian Polo-like family is Plk1. Plk1 specifically localizes to centrosomes, the spindle midzone and the post-mitotic bridge, and it participates in both mitotic entry and mitotic progression [22,23]. Depletion of *Plk1* by RNA interference results in metaphase arrest and formation of abnormal chromatin structures. Knockdown of *Plk1* also reduces cell survival and elevates drug sensitivity of tumor, but not normal, cells [24,25]. *Plk1* is overexpressed in a broad spectrum of cancer types, and its expression often correlates with poor prognosis. Thus, inhibition of Plk1 kinase activity might represent a promising approach for the development of novel anticancer therapies [23].

The other members of the Plk family, Plk2, Plk3 and Plk4, have been less studied. Plk2 and Plk3 were identified in response to mitogenic stimulation. However, they are possibly involved in other functions such as DNA damage checkpoint response or apoptosis [22]. Indeed, *Plk2* is a target of p53 and participates in the G_2 checkpoint and in modulation of apoptosis. Recently, *Plk2* has been found to be silenced by hypermethylation in B-cell neoplasia, suggesting a tumor suppressor role in this disease [26]. Plk4, the most divergent Plk family member, is involved in centrosome separation and mitotic fidelity [27]. Surprisingly, this kinase appears to be a tumor suppressor, because *Plk4* heterozygous mice develop liver and lung tumors due to high frequency of mitotic errors (Table 2) [28••]. These tumors do not lose the normal

allele, indicating that *Plk4* is haploinsufficient for tumor suppression [28••]. These findings indicate that Plks might have opposite roles in controlling cell division. Thus, therapeutic strategies against Plk1 must be highly selective to avoid those side-effects likely to result from inhibiting other Plks.

NIMA-related kinases

The NIMA (Never-in-mitosis *Aspergillus*)-related kinases (Nek) consist of eleven proteins with limited homology to the NIMA kinase of *Aspergillus nidulans* [29]. Some family members, such as Nek2, Nek6, Nek7 and Nek9, are involved in mitotic progression. Nek1 and Nek8, by contrast, might play a role in cilium formation, because they are mutated in polycystic kidney disease. Finally, Nek1, Nek2 and Nek11 participate in the DNA damage response [29]. Nek2 exhibits the greatest sequence identity to NIMA and is the best known member of the family. Nek2 has been implicated in SAC and in centriole function in cooperation with Plk1 [30,31]. Nek2 protein levels peak during S and G_2 phases, but are degraded by the proteasome after recognition by the anaphase-promoting complex (APC). Interestingly, targeting of Nek2 is independent of the APC adaptor proteins Cdc20 and Frizzled-related 1 (Fzr1), suggesting that degradation of Nek2 is uncoupled to SAC [32•]. Nek2 is overexpressed in a variety of human tumors and might be responsible for the appearance of multiple centrosomes and aneuploidy. Depletion of Nek2 prevents centrosome separation, delays mitosis and results in increased apoptosis, possibly as a result of mitotic errors, suggesting possible therapeutic uses in cancer [30].

The SAC kinases Bub1, BubR1 and Mps1

Bub1 and BubR1 (encoded by the *Bub1B* gene) are involved in the regulation of the chromosome-spindle attachment by SAC [13,33]. They are mutated in several colorectal, lung, breast and hematopoietic malignancies [13]. Biallelic mutations of the *Bub1B* gene have also been found in mosaic variegated aneuploidy syndrome and premature chromatid separation syndrome [34••,35••]. These rare autosomal recessive disorders are characterized by growth retardation, microcephaly and childhood cancer. These mutations result in lost or reduced checkpoint function, suggesting a role for these kinases in protecting from genomic instability. Deletion of *Bub1B* in the mouse germline results in early embryonic lethality. Heterozygous animals display increased chromosome instability accompanied by early aging phenotypes and increased cancer susceptibility (Table 1) [36••,37••]. Knockdown of *BubR1* or inhibition of its kinase activity in human cancer cells results in strong genomic abnormalities, massive chromosome loss and apoptotic cell death in a few cell divisions, suggesting potential therapeutic uses [38].

Mps1 is the mammalian homologue of the yeast Mps1p, a kinase involved in the regulation of APC during SAC [39]. Mps1 is a target of APC that controls Mps1 protein levels during mitosis, suggesting the existence of an Mps1–APC feedback circuit to irreversibly inactivate the SAC during anaphase [40]. Recent results indicate that Mps1 phosphorylates the Bloom syndrome protein Blm, a helicase required for genome stability [41]. Blm phosphorylation by Mps1 primes it for interaction with Plk1, which modulates Blm function. Although no mutations in *MPS1* have been found in human cancer, a hypomorphic Mps1 in zebrafish causes meiotic errors, aneuploidy and developmental defects [42], suggesting a relationship between this kinase and genomic stability. Inhibition of the yeast Mps1p by cincreasin [43•], a novel small molecule inhibitor, causes lethal chromosome mis-segregation only in mutants that display chromosomal instability as a result of defective SAC. An unrelated Mps1 inhibitor, SP600125, previously known as a Jnk (Janus kinase) inhibitor, cooperates with spindle poisons in triggering apoptosis due to aberrant mitotic progression [44•]. These observations have raised the possibility that Mps1, and possibly Bub kinases, can be exploited as cancer targets by selectively killing cancer cells that display chromosomal instability.

Conclusions

Abnormal cell division is one of the hallmarks of cancer cells. Evidence accumulated during the 90s pointed at misregulation of the early phases of the cell cycle as one of the pathways most frequently mutated in human cancer [2]. Many of these mutations result in Cdk misregulation, thus making these targets likely candidates for the development of selective inhibitors. Unfortunately, most current Cdk inhibitors have not been successful in the clinic, mostly owing to undesired side effects. Genetic evidence in mice indicates that interphase Cdks are not essential for the basic cell cycle but for proper homeostasis of specific cell types [4]. These findings are making it possible to genetically interrogate the therapeutic potential of these kinases in animal tumor models. Emerging evidence indicates that, whereas Cdk4 and/or Cdk6 kinase activity might be essential for HER2-driven mammary tumorigenesis, Cdk2 is dispensable for pituitary tumors induced by loss of p27Kip1 [10]. This information should be useful to design more rational therapeutic strategies based on selective targeting of individual Cdks in specific tumor types (Table 1).

Although aneuploidy has for decades been known to be a landmark of cancer cells, it is only recently that mitotic kinases have been implicated in cancer development. Some of these kinases such as Aurora kinases and Plk1 could be direct targets for therapeutic intervention [16,23]. Others, such as Mps1 and Bub, might prove to be novel therapeutic strategies that take advantage of the genetic instability of cancer cells to trigger apoptosis under conditions in which normal cells should not be affected. As discussed above for the Cdks, the success of

these novel therapeutic approaches will require a more detailed interrogation of the role of the mitotic kinases in genetically defined preclinical tumor models.

Acknowledgements

Work in our laboratories is supported by grants from the Plan Nacional de Investigación Científica to MM (BMC2003-06098) and MB (SAF2000-0009-CO2-01, SAF2002-10374-E, SAF1999-1825-CE and SAF2004-20477-E), 5th Framework Programme of the European Union to MB (QLK3-1999-00875, QLG2-CT-2002-00930, LSHC-CT-2004-503438) and Fundación Científica of the Asociación Española contra el Cáncer and Fundación Mutua Madrileña Automovilística to MM.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Futreal PA, Coin L, Marshall M, Down T, Hubbard T, Wooster R, Rahman N, Stratton MR: **A census of human cancer genes**. *Nat Rev Cancer* 2004, **4**:177-183.

2. Malumbres M, Barbacid M: **To cycle or not to cycle: a critical decision in cancer**. *Nat Rev Cancer* 2001, **1**:222-231.

3. Shapiro GI: **Cyclin-dependent kinase pathways as targets for cancer treatment**. *J Clin Oncol* 2006, **24**:1770-1783.

4. Malumbres M, Barbacid M: **Mammalian cyclin-dependent kinases**. *Trends Biochem Sci* 2005, **30**:630-641.

5. Chen HH, Wang YC, Fann MJ: **Identification and characterization of the CDK12/cyclin L1 complex involved in alternative splicing regulation**. *Mol Cell Biol* 2006, **26**:2736-2745.

6. Yu Q, Geng Y, Sicinski P: **Specific protection against breast cancers by cyclin D1 ablation**. *Nature* 2001, **411**:1017-1021.

7. •• Landis MW, Pawlyk BS, Li T, Sicinski P, Hinds PW: **Cyclin D1-dependent kinase activity in murine development and mammary tumorigenesis**. *Cancer Cell* 2006, **9**:13-22.
See annotation [9••].

8. •• Reddy HK, Mettus RV, Rane SG, Grana X, Litvin J, Reddy EP: **Cyclin-dependent kinase 4 expression is essential for neu-induced breast tumorigenesis**. *Cancer Res* 2005, **65**:10174-10178.
See annotation [9••].

9. •• Yu Q, Sicinska E, Geng Y, Ahnstrom M, Zagozdzon A, Kong Y, Gardner H, Kiyokawa H, Harris LN, Stal O *et al.*: **Requirement for CDK4 kinase function in breast cancer**. *Cancer Cell* 2006, **9**:23-32.
The studies by Landis *et al*. [7••], Reddy *et al*. [8••] and Yu *et al*. [9••] use two complementary approaches to analyze the requirement for Cdk4 kinase activity in mammary glands and breast tumorigenesis. Whereas Cdk4 kinase activity is dispensable for normal mammary gland development, ErbB2-induced breast tumors do not progress in the absence of Cdk4 kinase activity, suggesting a therapeutic use for Cdk4 inhibitors in ErbB2-positive breast cancer.

10. Malumbres M, Barbacid M: **Is Cyclin D1–CDK4 kinase a *bona fide* cancer target?** *Cancer Cell* 2006, **9**:2-4.

11. • Martin A, Odajima J, Hunt SL, Dubus P, Ortega S, Malumbres M, Barbacid M: **Cdk2 is dispensable for cell cycle inhibition and tumor suppression mediated by p27(Kip1) and p21(Cip1)**. *Cancer Cell* 2005, **7**:591-598.
See annotation [12•].

12. • Aleem E, Kiyokawa H, Kaldis P: **Cdc2-cyclin E complexes regulate the G1/S phase transition**. *Nat Cell Biol* 2005, **7**:831-836.
The studies by Martin *et al*. [11•] and Aleem *et al*. [12•] report that ablation of *Cdk2* has no effect on tumor development induced by *p27Kip1* deficiency, thus, suggesting that the tumor suppressor activity of this inhibitor in mediated by another kinase, possibly Cdk1.

13. Kops GJ, Weaver BA, Cleveland DW: **On the road to cancer: aneuploidy and the mitotic checkpoint**. *Nat Rev Cancer* 2005, **5**:773-785.

14. Vassilev LT, Tovar C, Chen S, Knezevic D, Zhao X, Sun H, Heimbrook DC, Chen L: **Selective small-molecule inhibitor reveals critical mitotic functions of human CDK1**. *Proc Natl Acad Sci USA* 2006, **103**:10660-10665.

15. Carmena M, Earnshaw WC: **The cellular geography of aurora kinases**. *Nat Rev Mol Cell Biol* 2003, **4**:842-854.

16. Keen N, Taylor S: **Aurora-kinase inhibitors as anticancer agents**. *Nat Rev Cancer* 2004, **4**:927-936.

17. Kanda A, Kawai H, Suto S, Kitajima S, Sato S, Takata T, Tatsuka M: **Aurora-B/AIM-1 kinase activity is involved in Ras-mediated cell transformation**. *Oncogene* 2005, **24**:7266-7272.

18. Tatsuka M, Sato S, Kitajima S, Suto S, Kawai H, Miyauchi M, Ogawa I, Maeda M, Ota T, Takata T: **Overexpression of Aurora-A potentiates HRAS-mediated oncogenic transformation and is implicated in oral carcinogenesis**. *Oncogene* 2005, **24**:1122-1127.

19. Eves EM, Shapiro P, Naik K, Klein UR, Trakul N, Rosner MR: **Raf kinase inhibitory protein regulates aurora B kinase and the spindle checkpoint**. *Mol Cell* 2006, **23**:561-574.

20. • Girdler F, Gascoigne KE, Eyers PA, Hartmuth S, Crafter C, Foote KM, Keen NJ, Taylor SS: **Validating Aurora B as an anti-cancer drug target**. *J Cell Sci* 2006, **119**:3664-3675.
The specific phenotype of inhibiting Aurora A or B using small molecules is analyzed. Inhibition of each kinase results in cell cycle arrest in different cell cycle phases, suggesting separate functions and distinct and independent therapeutic value for these two kinases.

21. •• Carter SL, Eklund AC, Kohane IS, Harris LN, Szallasi Z: **A signature of chromosomal instability inferred from gene expression profiles predicts clinical outcome in multiple human cancers**. *Nat Genet* 2006, **38**:1043-1048.
The analysis of gene expression profiles in a variety of human tumors suggests an expression signature linked to chromosomal instability and poor prognosis. Many of the genes represented in this signature are mitotic regulators, including several kinases such as Cdk1, Aurora A, Aurora B, Nek2 and Mps1.

22. Barr FA, Sillje HH, Nigg EA: **Polo-like kinases and the orchestration of cell division**. *Nat Rev Mol Cell Biol* 2004, **5**:429-440.

23. Strebhardt K, Ullrich A: **Targeting polo-like kinase 1 for cancer therapy**. *Nat Rev Cancer* 2006, **6**:321-330.

24. Spankuch B, Heim S, Kurunci-Csacsko E, Lindenau C, Yuan J, Kaufmann M, Strebhardt K: **Down-regulation of Polo-like kinase 1 elevates drug sensitivity of breast cancer cells *in vitro* and *in vivo***. *Cancer Res* 2006, **66**:5836-5846.

25. Liu X, Lei M, Erikson RL: **Normal cells, but not cancer cells, survive severe Plk1 depletion**. *Mol Cell Biol* 2006, **26**:2093-2108.

26. Syed N, Smith P, Sullivan A, Spender LC, Dyer M, Karran L, O'Nions J, Allday M, Hoffmann I, Crawford D *et al.*: **Transcriptional silencing of Polo-like kinase 2 (SNK/PLK2) is a frequent event in B-cell malignancies**. *Blood* 2006, **107**:250-256.

27. Habedanck R, Stierhof YD, Wilkinson CJ, Nigg EA: **The Polo kinase Plk4 functions in centriole duplication**. *Nat Cell Biol* 2005, **7**:1140-1146.

28. •• Ko MA, Rosario CO, Hudson JW, Kulkarni S, Pollett A, Dennis JW, Swallow CJ: **Plk4 haploinsufficiency causes mitotic infidelity and carcinogenesis**. *Nat Genet* 2005, **37**:883-888.
This work reports the result of partially inhibiting a cell cycle kinase involved in centrosome function and mitotic fidelity. Deficiency in *Plk4* provokes centrosomal amplification, multipolar spindles and aneuploidy, finally resulting in liver and lung tumors.

29. Quarmby LM, Mahjoub MR: **Caught Nek-ing: cilia and centrioles**. *J Cell Sci* 2005, **118**:5161-5169.

30. Hayward DG, Fry AM: **Nek2 kinase in chromosome instability and cancer**. *Cancer Lett* 2006, **237**:155-166.

31. Rapley J, Baxter JE, Blot J, Wattam SL, Casenghi M, Meraldi P, Nigg EA, Fry AM: **Coordinate regulation of the mother centriole component Nlp by Nek2 and Plk1 protein kinases**. *Mol Cell Biol* 2005, **25**:1309-1324.

32. • Hayes MJ, Kimata Y, Wattam SL, Lindon C, Mao G, Yamano H, Fry AM: **Early mitotic degradation of Nek2A depends on Cdc20-independent interaction with the APC/C**. *Nat Cell Biol* 2006, **8**:607-614.
This work analyzes the targeting of Nek2 by APC for proteasome-dependent degradation. Interestingly, binding of Nek2 occurs by a new mechanism independent of the APC adaptors Cdc20 and Fzr1, suggesting that the control of Nek2 protein levels is not linked to the spindle assembly checkpoint.

33. Lampson MA, Kapoor TM: **The human mitotic checkpoint protein BubR1 regulates chromosome-spindle attachments**. *Nat Cell Biol* 2005, **7**:93-98.

34. •• Hanks S, Coleman K, Reid S, Plaja A, Firth H, Fitzpatrick D, Kidd A, Mehes K, Nash R, Robin N *et al.*: **Constitutional aneuploidy and cancer predisposition caused by biallelic mutations in *BUB1B***. *Nat Genet* 2004, **36**:1159-1161.
See annotation [37••].

35. •• Matsuura S, Matsumoto Y, Morishima K, Izumi H, Matsumoto H, Ito E, Tsutsui K, Kobayashi J, Tauchi H, Kajiwara Y *et al.*: **Monoallelic *BUB1B* mutations and defective mitotic-spindle checkpoint in seven families with premature chromatid separation (PCS) syndrome**. *Am J Med Genet A* 2006, **140**:358-367.
See annotation [37••].

36. •• Baker DJ, Jeganathan KB, Cameron JD, Thompson M, Juneja S, Kopecka A, Kumar R, Jenkins RB, de Groen PC, Roche P *et al.*: ***BubR1* insufficiency causes early onset of aging-associated phenotypes and infertility in mice**. *Nat Genet* 2004, **36**:744-749.
See annotation [37••].

37. •• Dai W, Wang Q, Liu T, Swamy M, Fang Y, Xie S, Mahmood R, Yang YM, Xu M, Rao CV: **Slippage of mitotic arrest and enhanced tumor development in mice with *BubR1* haploinsufficiency**. *Cancer Res* 2004, **64**:440-445.
This series of studies [34••–37••] report the link between *BubR1* mutation and cancer development. The analysis of patients with variegated aneuploidy syndrome or premature chromatid separation syndrome revealed a wide spectrum of biallelic mutations in the *Bub1B* gene, which encodes the BubR1 kinase, suggesting that the deficiency in this kinase is linked to cancer susceptibility. Similarly, insufficiency in *BubR1* results in aging-associated phenotypes, and increased tumor susceptibility in the mouse.

38. Kops GJ, Foltz DR, Cleveland DW: **Lethality to human cancer cells through massive chromosome loss by inhibition of the mitotic checkpoint**. *Proc Natl Acad Sci USA* 2004, **101**:8699-8704.

39. Fisk HA, Mattison CP, Winey M: **A field guide to the Mps1 family of protein kinases**. *Cell Cycle* 2004, **3**:439-442.

40. Palframan WJ, Meehl JB, Jaspersen SL, Winey M, Murray AW: **Anaphase inactivation of the spindle checkpoint**. *Science* 2006, **313**:680-684.

41. Leng M, Chan DW, Luo H, Zhu C, Qin J, Wang Y: **MPS1-dependent mitotic BLM phosphorylation is important for chromosome stability**. *Proc Natl Acad Sci USA* 2006, **103**:11485-11490.

42. Poss KD, Nechiporuk A, Stringer KF, Lee C, Keating MT: **Germ cell aneuploidy in zebrafish with mutations in the mitotic checkpoint gene *mps1***. *Genes Dev* 2004, **18**:1527-1532.

43. • Dorer RK, Zhong S, Tallarico JA, Wong WH, Mitchison TJ, Murray AW: **A small-molecule inhibitor of Mps1 blocks the spindle-checkpoint response to a lack of tension on mitotic chromosomes**. *Curr Biol* 2005, **15**:1070-1076.
See annotation [44•].

44. • Schmidt M, Budirahardja Y, Klompmaker R, Medema RH: **Ablation of the spindle assembly checkpoint by a compound targeting Mps1**. *EMBO Rep* 2005, **6**:866-872.
The studies by Dorer *et al*. [43•] and Schmidt *et al*. [44•] identified Mps1 as one of the crucial targets of new cell cycle inhibitors that are especially effective on cells with alterations in the spindle checkpoint, suggesting therapeutic uses against spindle checkpoint-deficient cancer cells.

45. Sotillo R, Dubus P, Martin J, de la Cueva E, Ortega S, Malumbres M, Barbacid M: **Wide spectrum of tumors in knock-in mice carrying a Cdk4 protein insensitive to INK4 inhibitors**. *EMBO J* 2001, **20**:6637-6647.

46. Sotillo R, Garcia JF, Ortega S, Martin J, Dubus P, Barbacid M, Malumbres M: **Invasive melanoma in Cdk4-targeted mice**. *Proc Natl Acad Sci USA* 2001, **98**:13312-13317.

47. Sotillo R, Renner O, Dubus P, Ruiz-Cabello J, Martin-Caballero J, Barbacid M, Carnero A, Malumbres M: **Cooperation between Cdk4 and p27kip1 in tumor development: a preclinical model to evaluate cell cycle inhibitors with therapeutic activity**. *Cancer Res* 2005, **65**:3846-3852.

48. Zhang D, Hirota T, Marumoto T, Shimizu M, Kunitoku N, Sasayama T, Arima Y, Feng L, Suzuki M, Takeya M *et al.*: **Cre-loxP-controlled periodic Aurora-A overexpression induces mitotic abnormalities and hyperplasia in mammary glands of mouse models**. *Oncogene* 2004, **23**:8720-8730.

49. Rao CV, Yang YM, Swamy MV, Liu T, Fang Y, Mahmood R, Jhanwar-Uniyal M, Dai W: **Colonic tumorigenesis in *BubR1*$^{+/-}$*Apc*$^{Min/+}$ compound mutant mice is linked to premature separation of sister chromatids and enhanced genomic instability**. *Proc Natl Acad Sci USA* 2005, **102**:4365-4370.