*Minireview*

# Targeting Aurora-2 Kinase in Cancer[1]

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## Abstract

**Aurora-2 kinase has been shown to contribute to oncogenic transformation and is frequently overexpressed and amplified in many human tumor types. Aurora-2 belongs to a small family of mitotic serine/threonine kinases that regulate centrosome maturation, chromosome segregation, and cytokinesis. The mechanism behind the transforming activity of aurora-2 is not fully understood; however, the role of aurora-2 in regulating the centrosome cycle is likely responsible for its ability to transform cells. Aurora-2 overexpression has been correlated with centrosome amplification, which can be a driving cause of genomic instability in tumor cells. In addition, recent work has demonstrated that aurora-2 plays an active function in promoting entry into mitosis by regulating local translation of centrosomal stored mRNA, such as cyclin B1. These recent findings implicate aurora-2 as an important regulator of both genomic integrity and cell cycle progression in cancer cells and suggest that aurora-2 is an attractive target for anticancer drug development.**

## Introduction

Advances in cancer research over the past 30 years have greatly increased our knowledge and understanding of the genetics of cancer, including the multistep basis of tumorigenesis (1). Simply stated, the multistep model suggests that a series of genetic alterations is necessary for the transformation of a normal cell into a cell with a malignant tumorigenic phenotype. Even though great progress has been made in identifying the genetic alterations responsible for the overall process, the underlying factors driving these genetic changes are not well understood. As we learn more about the abnormal physiology of cancer cells and about the underlying causes of these abnormalities, we should be able to find new targets for therapies that specifically affect cancer cells.

Two distinguishing characteristics of most cancer cells are (*a*) a change in the amount or organization of DNA compared with normal cells and (*b*) the loss of control of key regulatory cell cycle checkpoints (2, 3). It is arguable whether these two characteristics are acquired during the early events of tumorigenesis and are largely responsible for late-stage cancer phenotypes. It is likely that cancer is a disease of the cell cycle, but there are additional characteristics that contribute to the overall cancer phenotype.

Changes in DNA amount are most commonly due to gains or losses of whole chromosomes (tumor cell aneuploidy). The causes of aneuploidy are not fully understood, but the improper segregation of chromosomes during mitosis may be an important factor (4). Precise segregation of chromosomes is an important event in the progression of the cell cycle and is a highly complex process. There are many potential causes of improper segregation during mitosis. Recently, much attention has been focused on the centrosome, which in normal cells appears to orchestrate the changes required for proper chromosome segregation (5). This orchestration includes: (*a*) serving as an anchor for microtubule nucleation; (*b*) serving as an organizing center for transformation of the microtubules into a mitotic spindle apparatus; and (*c*) serving as a platform for recruitment of structural, motor, and catalytic proteins that constitute the centrosome complex (6). One set of centrosome-associated proteins is the family of kinases called AIRKs,[3] which are thought to be key regulators of centrosome duplication, chromosome segregation, and cytokinesis (7). The nomenclature of AIRK family members in various organisms is complicated and sometimes confusing. Table 1 shows a summary of AIRK family members and the nomenclature found throughout the scientific literature.

An additional function of AIRKs, which is just beginning to be understood, is their role as regulators of entry into and exit from mitosis. This putative function is based primarily on pioneering work in *Xenopus*, where it has been shown that Eg2, a *Xenopus* AIRK, is responsible for signaling entry into the $G_2$-M phase of meiosis II in developing oocytes, through control of the expression of cell cycle-regulatory proteins in a transcription-independent manner (8, 9). Considering this finding, it is tempting to speculate a role for AIRKs in regulating entry into mitosis in mammalian cells as well, thereby suggesting an additional role for AIRKs in tumorigenesis. Combined with the role that AIRKs play in controlling the integrity of chromosome separation, this implicates AIRK family members as possible components that provide the underlying driving force in the progression of tumorigenesis. The remainder of this review will focus on describing the

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[3] The abbreviations used are: AIRK, Aurora/IP11p-related kinase; CPEB, cytoplasmic polyadenylation element-binding factor.

*Table 1* AIRKs in various organisms

| Designation | Organism | Other designation | Ref. no. |
|---|---|---|---|
| Ipllp | *Saccharomyces cerevisiae* | | 47 |
| Aurora | *Drosophila melanogaster* | | 15 |
| Aurora-B | *D. melanogaster* | IAL | 48 |
| Eg2 | *Xenopus laevis* | | 16 |
| AIR-1 | *Caenorhabditis elegans* | | 49 |
| AIR-2 | *C. elegans* | STU-7 | 50 and 51 |
| AIM | *Rattus norvegicus* | | 52 |
| ARK-1 | *Mus musculus* | IAK1, Ayk1 | 53 |
| ARK-2 | *M. musculus* | | 53 |
| AIE1 | *M. musculus* | | 21 |
| Aurora-1 | *Homo sapiens* | Aurora B, AIK2, ARK2, STK12 | 11, 44, and 53 |
| Aurora-2 | *H. sapiens* | Aurora A, AIK1, ARK1, STK15, BTAK | 11, 13, 26, and 53 |
| Aurora-3 | *H. sapiens* | Aurora C, AIK3, STK13, AIE2 | 19–21 |

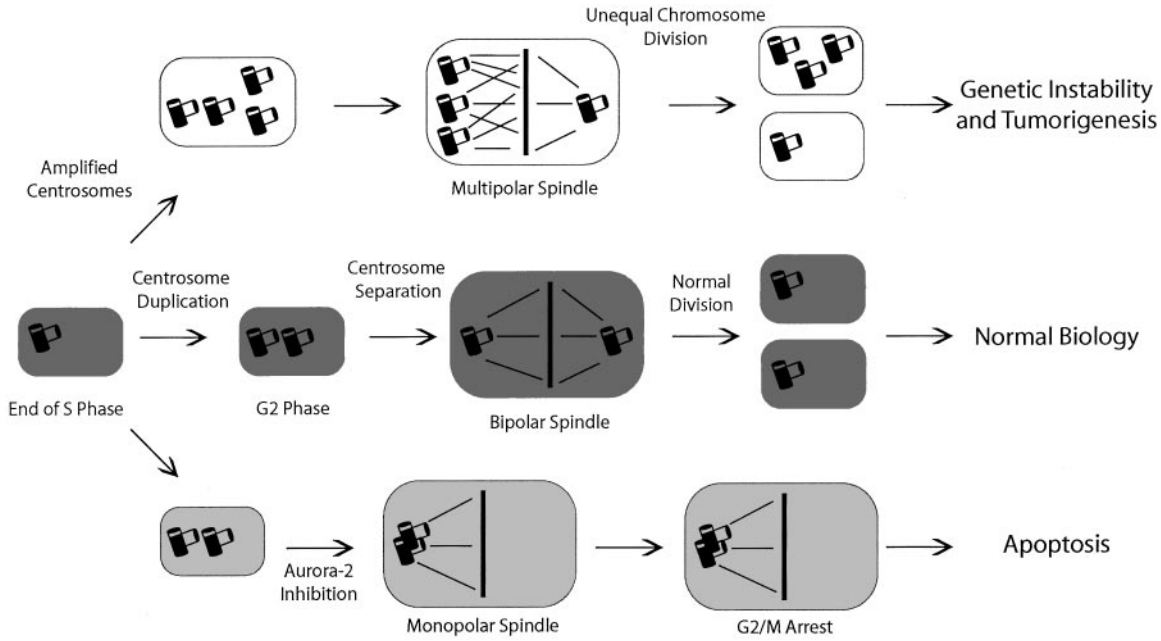


*Fig. 1*. Aurora-2 kinase is a regulator of centrosome duplication and separation. Under normal biological conditions, this results in equal segregation of chromosomes during mitosis. Aurora-2 overexpression causes centrosome amplification and the formation of multipolar mitotic spindles, which leads to aneuploidy and tumorigenesis. Inhibition of aurora-2 results in cell cycle arrest and apoptosis.

oncogenic role that AIRKs (specifically aurora-2) play in tumorigenesis as regulators of both centrosome function and direct mitotic control.

## AIRKs

AIRKs are serine/threonine kinases that show conservation of both structure and function throughout eukaryotic organisms. AIRKs have a COOH-terminal catalytic domain that is highly conserved within the family and an $NH_2$-terminal domain that is variable among organisms (10). The most conserved motif is the putative activation loop, where many protein kinases are activated by phosphorylation. The activation loop contains a highly conserved threonine residue that is likely phosphorylated by regulatory kinases. Mutating this threonine ($Thr^{288}$ in aurora-2) to an acidic residue results in an enzyme with hyperactivity (11), suggesting that the threonine residue is a target for kinase activation.

The AIRKs are associated with centrosome and microtubule dynamics. They localize to the centrosome around the pericentriolar material, microtubules, and the mitotic spindle, in keeping with their role in chromosome segregation and cell division (12). A temperature-sensitive mutant of the *Saccharomyces cerevisiae* yeast AIRK, Ipl1, demonstrates abnormal chromosome number, indicating that Ipl1 kinase regulates chromosome segregation events (10).

Three AIRK genes have been identified in *Homo sapiens*. The first human AIRK family member is *aurora-2*, also known as *aurora A*, *aik1*, *btak*, *stk15*, and *ark1* (Refs. 11–13; Table 1). *Aurora-2* maps to chromosome 20q13.2-q13.3, a region frequently amplified in human malignant tumors (14), and its

*Table 2* Reported aurora-2 kinase abnormalities in human tumors

| Type of human tumor | Type of Specimen | Findings | Ref. no. |
|---|---|---|---|
| Colorectal cancer | Primary | Overexpressed in >50% | 11 |
| Breast cancer | Primary | Amplified in 12% | 26 |
| | | Overexpression in 94% | 54 |
| Gastric cancer | Primary | Amplified in 5% and overexpressed in >50% | 55 |
| Bladder cancer | Primary | Amplification is associated with aneuploidy and tumor aggressiveness | 56 |
| Pancreatic cancer | Primary | Overexpressed in 93% | 23 |
| | | Overexpressed in 53% | 24 |
| Breast, ovarian, colon, prostate, neuroblastoma, cervical, and pancreatic cancers | Cell lines | Both overexpression and amplification noted | 12, 22–24, and 26 |

protein product localizes to the centrosome and spindle poles (12). Specific inhibition of aurora-2 kinase by mutation leads to formation of a monopolar mitotic spindle, indicating its requirement for centrosome distribution and formation of the bipolar spindle (Refs. 15 and 16; Fig. 1). A second human AIRK gene is *aurora-1*, also called *aurora B*, *aik2*, *ark2*, and *stk12* (11, 17), which maps to chromosome 17p13 and encodes a protein that localizes to the midbody of the microtubules. The inhibition of aurora-1 kinase results in the formation of multinucleated cells, suggesting a role in cytokinesis (18). The third human gene is aurora-3, also referred to as *aurora C*, *aie2*, and *aik3* (19, 20), which maps to chromosome 19q13.3. Its gene product localizes to the centrosome only during anaphase (10). The expression of aurora-3 kinase has been shown to be testis specific (21).

## Aurora-2 Kinase in Tumorigenesis

AIRKs are key regulators of centrosome maturation, chromosome segregation, and cytokinesis. Of the three human AIRKs, aurora-2 has been most strongly implicated in carcinogenesis. Aurora-2 is overexpressed and/or amplified in various malignancies such as primary colorectal, breast, and gastric tumors as well as breast, ovarian, colon, prostate, neuroblastoma, cervical, and gastric cancer cell lines (Table 2). In addition to being overexpressed, the gene encoding aurora-2 kinase is often amplified in many tumor types, suggesting one possible mechanism for its overexpression (11, 13). Using microarray studies, we recently showed that aurora-2 is overexpressed and its gene is amplified in human pancreatic tumors and pancreatic cancer cell lines (Refs. 22 and 23; Fig. 2); this has recently been confirmed by others (24). Aurora-2 kinase is of considerable interest as a potential drug target because it shows both gene amplification and up-regulation in cancer tissue. These findings are quite reminiscent of the situation in the early exploration of HER-2/neu as a target in breast cancer in the mid-1980s (25).

Aurora-2 kinase has been shown to play an oncogenic role in cell culture studies (11). Overexpression of aurora-2 transforms rat fibroblasts, and ectopic expression of aurora-2 in mouse NIH 3T3 cells leads to transformation *in vitro* and to the appearance of abnormal centrosome numbers (26). Overexpression of aurora-2 kinase in near-diploid human breast epithelial cells results in centrosome abnormalities with induction of aneuploidy (26). Overexpression of aurora-2 and centrosome amplification have been shown to be early

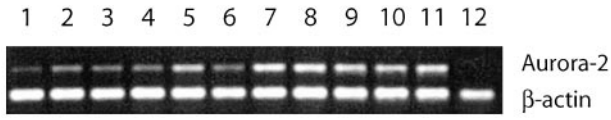


*Fig. 2*. RT-PCR showing the overexpression of aurora-2 in a panel of 11 pancreatic cancer cell lines (*Lanes 1–11*) and normal pancreas (*Lane 12*). The *top band* in each lane is the product of aurora-2, and the *bottom band* is a β-actin product included as an internal control. DNA was resolved on a 1% agarose gel and visualized by ethidium bromide staining. *Lane 1*, AsPC-1; *Lane 2*, BxPC-3; *Lane 3*, Capan-1; *Lane 4*, Capan-2; *Lane 5*, CFPAC-1; *Lane 6*, HPAF II; *Lane 7*, Hs766T; *Lane 8*, Mia PaCa-2; *Lane 9*, Mutj; *Lane 10*, PANC-1; *Lane 11*, SU.86.86; *Lane 12*, normal pancreas.

events in tumorigenesis in a rat mammary carcinogenesis model (27). These findings lend support to the hypothesis that aurora-2 amplification and overexpression help drive the multistep acquisition of genetic alterations required for tumorigenesis. In this review, we propose that the oncogenicity of aurora-2 results from two related yet distinct functions of the protein, namely, (*a*) chromosome segregation and control of genomic stability and (*b*) regulation of entrance into mitosis. The remainder of the review will discuss the involvement of aurora-2 in these processes and how changes in aurora-2 expression might lead to tumorigenesis.

## Aurora-2 in Genomic Instability

Genomic instability, manifested by changes in the amount and organization of DNA compared with normal cells (2), has proven to be a hallmark of most cancer cells. Genomic instability is grouped into four categories of DNA alterations: (*a*) subtle DNA sequence changes; (*b*) whole gains and losses of chromosomes; (*c*) chromosome translocations; and (*d*) gene amplifications (28). The molecular mechanisms responsible for mediating each type of genomic instability are now beginning to be understood. The most common manifestation of genomic instability in cancer cells involves the gain or loss of whole chromosomes, resulting in aneuploidy. In colorectal cancers, for example, aneuploidy occurs 10–100 times more frequently than any other type of genomic instability (29). The causes and outcomes of aneuploidy are still areas of active investigation and disagreement. As shown in many tumor types, problems in chromosome segregation can be caused by improper mitotic checkpoint function (4), allowing a cell to proceed with mitosis before formation of the

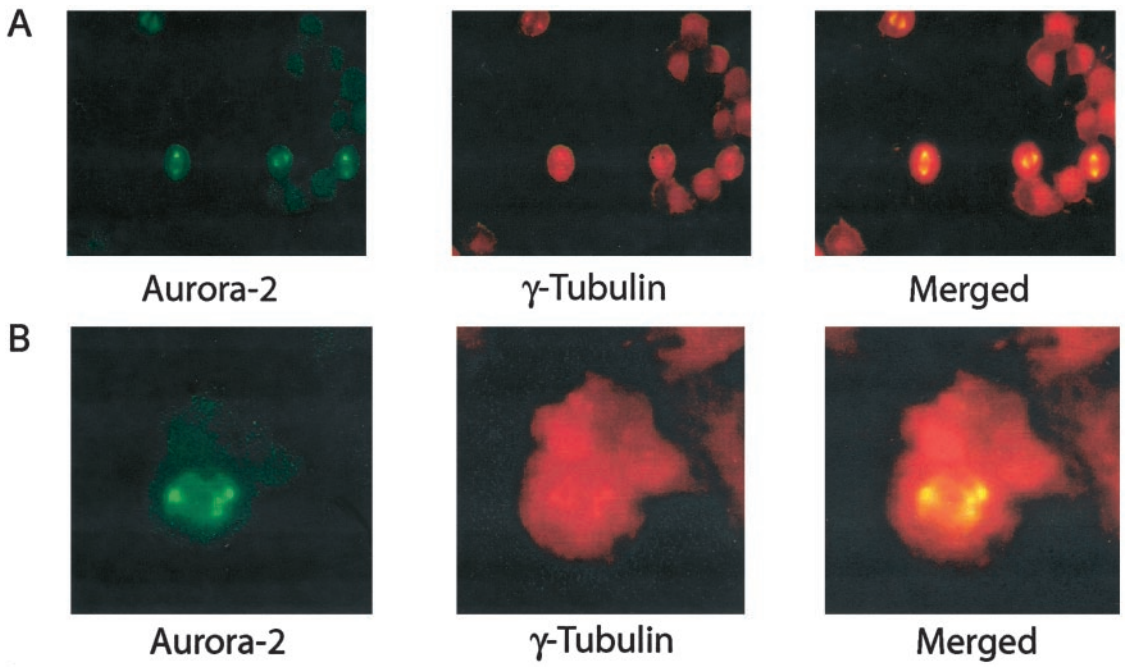


*Fig. 3*. As shown by immunofluorescence microscopy, aurora-2 kinase is associated with the centrosome and plays an important role in centrosome function and microtubule dynamics. In both normal (*A*) and cancer (*B*) cells, aurora-2 protein colocalizes with γ-tubulin, a centrosome marker. The *third images* (*Merged*) in *A* and *B* represent an overlay of the first two images. Overexpression of aurora-2 leads to increases in centrosome numbers and formation of abnormal mitotic spindles (*B*) as seen in the pancreatic cancer cell line Mia PaCa-2.

appropriate mitotic machinery. A further cause of aneuploidy involves the loss of regulation of centrosome duplication and function, resulting in cells with abnormal centrosome numbers (4).

A key aspect of chromosome segregation during mitosis is the duplication of the centrosome and the organization of the mitotic spindle poles. A centrosome is typically composed of two centrioles encompassed by a relatively undefined electron-dense pericentriolar material. A centriole is composed of a nine-triplet array of microtubules made up of α-, β-, δ-, and ϵ-tubulin together with centrin proteins (30). The pericentriolar material is a network of filamentous polymers speckled with small ring-shaped structures of γ-tubulin. γ-Tubulin is believed to serve as the nucleation site for microtubule elongation (31).

There are numerous stages during centrosome duplication and function that can be regulated. During the $G_1$ phase of the cell cycle, the two centrioles of a single centrosome remain together to function as a single unit, and then they separate into two components at the onset of S phase. The individual centrioles give rise to new centriole partners, resulting in two distinct centrosomes capable of microtubule assembly and mitotic spindle organization. Centrosomes organize microtubule polymerization during interphase; however, during mitosis, there is a dramatic increase in microtubule nucleation potential. The increase in microtubule nucleation is believed to assure the random contact between centrosome microtubules and chromosome kinetochores (32). Upon establishment of the mitotic spindle, the spindle microtubules shorten, and sister chromatids segregate and move toward opposite spindle poles. Motor proteins such as dynein (33) and kinesins are involved in the dynamic behavior of the mitotic spindle (34, 35). Furthermore, phosphorylation or dephosphorylation of key centrosomal proteins has been shown to regulate the rate of microtubule assembly and the organization of the mitotic spindle (36). The precise mechanism by which the centrosomal cycle or microtubule assembly and disassembly is regulated has not been fully elucidated, but members of the aurora family appear to play central roles in the entire process.

Whereas the precise role that aurora-2 plays in centrosome duplication has yet to be determined, some interesting observations have recently been made. Overexpression of aurora-2 in cell culture has been shown to cause centrosome amplification (Fig. 3), although it appears to do so in a kinase-independent manner, as evidenced by the fact that overexpression of a kinase-dead mutant of aurora-2 in cell culture still results in an amplified centrosome phenotype (37). However, aurora-2 kinase activity is necessary to obtain a transformed phenotype by overexpression of aurora-2. Therefore, in addition to its role in centrosome duplication, genetic and biochemical studies have shown that aurora-2 kinase activity is crucial for proper centrosome separation (15). Again, the precise mechanism is not understood, but aurora-2 has been shown to phosphorylate substrates thought to be involved in centrosome separation, such as the kinesin-related protein Eg5 in *Xenopus* (38). Other specific signaling pathways that involve tightly regulated phosphorylation events involving aurora-2 remain unknown, and determining protein-protein

interactions with aurora-2 is currently an active area of investigation.

## Aurora-2 in Mitotic Control

As shown in *Xenopus laevis* oocytes, aurora family members play an important role in regulating the entry into mitosis (8). Oocyte development is halted at the $G_2$-M transition of meiosis II, where it rests until maturation is triggered by the steroid hormone progesterone. Progesterone binds to an unknown surface-associated receptor and signals the resumption of the meiotic cell cycle without the expression of newly transcribed genes. This "reawakening" is achieved by the translation of mRNAs that have been stored in the oocytes since the meiotic arrest. The stored mRNAs have short poly(A) tails and are not translated until modified to have an extended poly(A) tail. The signaling pathway proposed for this process begins with the activation of the *Xenopus* aurora kinase, Eg2, after progesterone binds its receptor. The activated Eg2 phosphorylates the CPEB, which in turn promotes the addition of a long poly(A) tail onto specific strands of mRNA, resulting in their translation (9).

This signaling scheme has also been shown in mouse oocytes (39). After the induction of oocyte maturation, ARK1, the murine equivalent of aurora-2, becomes active and phosphorylates CPEB on $Ser^{174}$. Either blocking ARK1 activity or truncating CPEB such that it cannot be modified by ARK1 inhibits cytoplasmic polyadenylation and leads to the inhibition of meiotic progression. Interestingly, cyclin B1 mRNA is a target for cytoplasmic polyadenylation during mouse oocyte maturation (39). The expression and formation of an active cdc2/cyclin B1 is known to be required for progression into mitosis. Thus, in summary, members of the aurora family appear to play essential roles in regulating meiosis and possibly meiotic progression in vertebrates.

Aurora-2 amplification and overexpression have been linked to cancer development and early progression for several years (11). However, the fact that aurora-2 continues to be overexpressed in late-stage tumors has been somewhat enigmatic. It is clear that up-regulation of aurora-2 results in abnormal mitotic spindles and gives rise to genomic instability and aneuploidy. This increases the rate of acquisition of the necessary genetic mutations that give rise to a cancer phenotype. However, after the genetic mutations are present, how do cancer cells cope with continued overexpression, and why does there appear to be a strong selective pressure to maintain aurora-2 overexpression in many tumor types?

We speculate that besides inducing genomic instability, aurora-2 and possibly other aurora family members play important roles in regulating the commencement of mitosis in somatic cells, in addition to their roles in developing oocytes. This could be accomplished by regulating the cytoplasmic polyadenylation of transcripts that code for key regulatory proteins such as cyclin B1 (Fig. 4). Consistent with this view, recent results indicate that cyclin B1 mRNA is polyadenylated in somatic cells before entry into mitosis (40) Additional mechanisms could be through the association of aurora-2 kinase with cell cycle-regulatory proteins such as the cdc2/cyclin B1 complex (41), p53 (42), the GTPase-activating protein (RasGAP; Ref. 43), or other protein interactions that have yet to be discovered. These functions would require aurora-2 kinase to be continuously expressed throughout tumorigenesis.

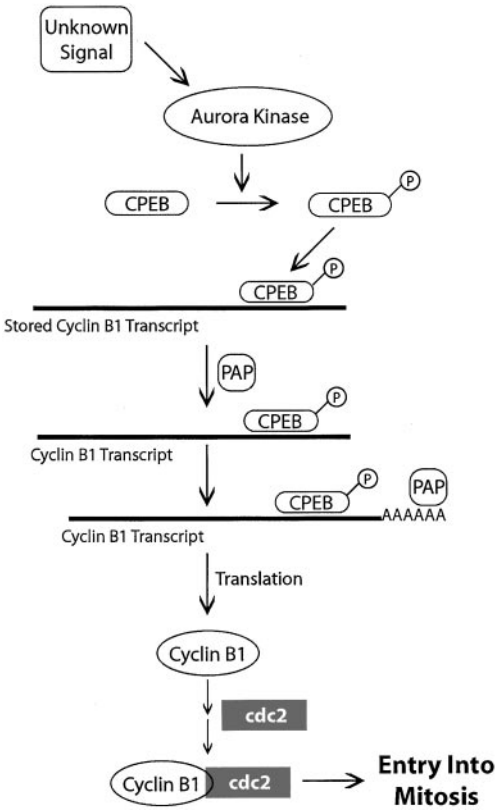


*Fig. 4*. Several recent studies have reported that aurora-2 kinase controls the translation of centrosomal stored transcripts, such as cyclin B1, by regulating the phosphorylation status of the CPEB. As illustrated above, a signal that has yet to be determined activates aurora-2 kinase, resulting in the phosphorylation of CPEB, which is bound to the 3′-untranslated region of stored unpolyadenylated messenger RNAs. The phospho-CPEB protein facilitates the necessary changes to recruit polyadenylation polymerase (*PAP*), which adds a poly(A) tail to the mRNA. Once polyadenylated, these transcripts are locally translated. This suggests that in addition to regulating the centrosome cycle, aurora-2 also plays an important role in controlling the entrance into mitosis in both normal and cancer cells by regulating the transcription-independent expression of cyclin B1.

## Aurora-2 as a Molecular Therapeutic Target

Taken together, the above findings make aurora-2 a potential therapeutic target of great interest. Significantly, aurora-2 appears to be largely and specifically up-regulated and amplified in cancer cells compared with normal cells. Northern blotting studies have shown the expression of some of the aurora kinase family members in normal tissues. More specifically, aurora-2 kinase has been described as high in thymus, with low levels in small bowel, testis, colon, spleen, and brain (44). Another study showed AIRKs to be elevated only in the testes (12). This information suggests that an inhibitor targeting aurora-2 kinase would have some selectivity for tumor cells *versus* normal cells.

A homology model and the crystal structure of aurora-2 have recently been published (45). The homology model was constructed based on sequence identity and homology to other serine/threonine kinases. In particular, aurora-2 showed good sequence alignment with cAMP-dependent kinase. The homology model was similar to the crystal structure obtained by X-ray crystallography using a truncated version of aurora-2 (residues 107–403; Ref. 46). The three-dimensional structure of aurora-2 reveals an ATP binding site that is relatively well conserved among other kinases. Despite the overall conservation, there are several unique features of the active site that can be exploited for drug design. Unique to the aurora family of kinases is the presence of two lysine residues in the glycine-rich nucleotide binding motif (45). Such differences may prove useful in designing inhibitors with aurora kinase selectivity. The solved structure confirms the unique conformation of the activation loop that appears to be conserved in AIRKs. Sequence and structural similarities among the three human AIRKs suggest it will be a challenge to design small molecules with absolute specificity for a single AIRK family member.

## Conclusions

The discovery that genomic instability plays a critical role in the onset and progression of human tumors has led to a search for factors controlling genomic stability. Of the four main categories of genomic instability, aneuploidy is most frequent and is a defining characteristic of most human cancers. One of the major mechanisms governing aneuploidy is the proper segregation of chromosomes during cell division. A family of serine threonine kinases that regulates the segregation of chromosomes has emerged as potential mediators of aneuploidy and therefore potential novel targets for antitumor drug development. The finding that aurora-2 kinase plays a role in oncogenic transformation further demonstrates that these kinases may be attractive potential drug targets.

The putative role that AIRKs play in regulating entry into and exit from mitosis suggests an additional mechanism behind the oncogenicity of aurora-2. It also provides a model to explain continued overexpression of aurora-2 after the development of a primary tumor. Cancer is partly defined by a cell's ability to continuously cycle through the mitotic process, therefore requiring the disruption of key regulatory proteins that monitor and signal the transitions required throughout the cell cycle. Based on known interactions of aurora kinase family members with cell cycle-regulatory proteins, such as cdc2/cyclin B1, it is probable that aurora-2 plays a central role in regulating the mitotic process.

Currently, several drug development efforts are under way to further validate aurora kinases as drug targets and to identify inhibitors for cancer therapy. It is too soon to know whether inhibitors of the aurora kinases will make good anticancer agents, but it is certain that these proteins represent new entry points for therapies targeting abnormal centrosome function in cancer.

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## References

1. Vogelstein, B., and Kinzler, K. W. The multistep nature of cancer. Trends Genet., *9:* 138–141, 1993.

2. Mitelman, F., Johansson, B., and Mertens, F. Catalog of Chromosome Aberrations in Cancer. New York: Wiley-Liss, 1994.

3. Zhou, B. B., and Elledge, S. J. The DNA damage response: putting checkpoints in perspective. Nature (Lond.), *408:* 433–439, 2000.

4. Sen, S. Aneuploidy and cancer. Curr. Opin. Oncol., *12:* 82–88, 2000.

5. Sakai, H. Microtubules in mitosis. Cell Struct. Funct., *19:* 57–62, 1994.

6. Kellogg, D. R., Moritz, M., and Alberts, B. M. The centrosome and cellular organization. Annu. Rev. Biochem., *63:* 639–674, 1994.

7. Gopalan, G., Chan, C. S., and Donovan, P. J. A novel mammalian, mitotic spindle-associated kinase is related to yeast and fly chromosome segregation regulators. J. Cell Biol., *138:* 643–656, 1997.

8. Andresson, T., and Ruderman, J. V. The kinase Eg2 is a component of the *Xenopus* oocyte progesterone-activated signaling pathway. EMBO J., *17:* 5627–5637, 1998.

9. Mendez, R., Hake, L. E., Andresson, T., Littlepage, L. E., Ruderman, J. V., and Richter, J. D. Phosphorylation of CPE binding factor by Eg2 regulates translation of c-mos mRNA. Nature (Lond.), *404:* 302–307, 2000.

10. Giet, R., and Prigent, C. Aurora/Ipl1p-related kinases, a new oncogenic family of mitotic serine-threonine kinases. J. Cell Sci., *112* (Pt. 21)*:* 3591–3601, 1999.

11. Bischoff, J. R., Anderson, L., Zhu, Y., Mossie, K., Ng, L., Souza, B., Schryver, B., Flanagan, P., Clairvoyant, F., Ginther, C., Chan, C. S., Novotny, M., Slamon, D. J., and Plowman, G. D. A homologue of *Drosophila* aurora kinase is oncogenic and amplified in human colorectal cancers. EMBO J., *17:* 3052–3065, 1998.

12. Kimura, M., Kotani, S., Hattori, T., Sumi, N., Yoshioka, T., Todokoro, K., and Okano, Y. Cell cycle-dependent expression and spindle pole localization of a novel human protein kinase, Aik, related to Aurora of *Drosophila* and yeast Ipl1. J. Biol. Chem., *272:* 13766–13771, 1997.

13. Sen, S., Zhou, H., and White, R. A. A putative serine/threonine kinase encoding gene BTAK on chromosome 20q13 is amplified and overexpressed in human breast cancer cell lines. Oncogene, *14:* 2195–2200, 1997.

14. Tanner, M. M., Grenman, S., Koul, A., Johannsson, O., Meltzer, P., Pejovic, T., Borg, A., and Isola, J. J. Frequent amplification of chromosomal region 20q12–q13 in ovarian cancer. Clin. Cancer Res., *6:* 1833–1839, 2000.

15. Glover, D. M., Leibowitz, M. H., McLean, D. A., and Parry, H. Mutations in aurora prevent centrosome separation leading to the formation of monopolar spindles. Cell, *81:* 95–105, 1995.

16. Roghi, C., Giet, R., Uzbekov, R., Morin, N., Chartrain, I., Le Guellec, R., Couturier, A., Doree, M., Philippe, M., and Prigent, C. The *Xenopus* protein kinase pEg2 associates with the centrosome in a cell cycle-dependent manner, binds to the spindle microtubules and is involved in bipolar mitotic spindle assembly. J. Cell Sci., *111* (Pt. 5)*:* 557–572, 1998.

17. Prigent, C., Gill, R., Trower, M., and Sanseau, P. *In silico* cloning of a new protein kinase, Aik2, related to *Drosophila* Aurora using the new tool: EST Blast. In Silico Biol., *1:* 123–128, 1999.

18. Tatsuka, M., Katayama, H., Ota, T., Tanaka, T., Odashima, S., Suzuki, F., and Terada, Y. Multinuclearity and increased ploidy caused by overexpression of the aurora- and Ipl1-like midbody-associated protein mitotic kinase in human cancer cells. Cancer Res., *58:* 4811–4816, 1998.

19. Bernard, M., Sanseau, P., Henry, C., Couturier, A., and Prigent, C. Cloning of STK13, a third human protein kinase related to *Drosophila* aurora and budding yeast Ipl1 that maps on chromosome 19q13.3-ter. Genomics, *53:* 406–409, 1998.

20. Kimura, M., Matsuda, Y., Yoshioka, T., and Okano, Y. Cell cycle-dependent expression and centrosome localization of a third human

aurora/Ipl1-related protein kinase, AIK3. J. Biol. Chem., *274:* 7334–7340, 1999.

21. Tseng, T. C., Chen, S. H., Hsu, Y. P., and Tang, T. K. Protein kinase profile of sperm and eggs: cloning and characterization of two novel testis-specific protein kinases (AIE1, AIE2) related to yeast and fly chromosome segregation regulators. DNA Cell Biol., *17:* 823–833, 1998.

22. Han, H., Bearss, D. J., Browne, L. W., Calaluce, R., Nagle, R. B., and Von Hoff, D. D. Identification of differentially expressed genes in pancreatic cancer cells using cDNA microarray. Cancer Res., *62:* 2890–2896, 2002.

23. Rojanala, S., Han, H., Munoz, R. M., Vankayalapati, H., Mahadevan, D., Hurley, L. H., Von Hoff, D. D., and Bearss, D. J. Aurora kinase-2, a potential molecular therapeutic target for pancreatic cancers. Proc. Am. Assoc. Cancer Res., *43:* 665, 2002.

24. Li, D., Zhu, J., Firozi, P. F., Abbruzzese, J. L., Evans, D. B., Cleary, K., Friess, H., and Sen, S. Overexpression of oncogenic STK15/BTAK/Aurora A kinase in human pancreatic cancer. Clin. Cancer Res., *9:* 991–997, 2003.

25. Slamon, D. J., Clark, G. M., Wong, S. G., Levin, W. J., Ullrich, A., and McGuire, W. L. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science (Wash. DC), *235:* 177–182, 1987.

26. Zhou, H., Kuang, J., Zhong, L., Kuo, W. L., Gray, J. W., Sahin, A., Brinkley, B. R., and Sen, S. Tumour amplified kinase STK15/BTAK induces centrosome amplification, aneuploidy and transformation. Nat. Genet., *20:* 189–193, 1998.

27. Goepfert, T. M., Adigun, Y. E., Zhong, L., Gay, J., Medina, D., and Brinkley, W. R. Centrosome amplification and overexpression of aurora A are early events in rat mammary carcinogenesis. Cancer Res., *62:* 4115–4122, 2002.

28. Lengauer, C., Kinzler, K. W., and Vogelstein, B. Genetic instabilities in human cancers. Nature (Lond.), *396:* 643–649, 1998.

29. Lengauer, C., Kinzler, K. W., and Vogelstein, B. Genetic instability in colorectal cancers. Nature (Lond.), *386:* 623–627, 1997.

30. Fuller, S. D., Gowen, B. E., Reinsch, S., Sawyer, A., Buendia, B., Wepf, R., and Karsenti, E. The core of the mammalian centriole contains γ-tubulin. Curr. Biol., *5:* 1384–1393, 1995.

31. Moritz, M., Braunfeld, M. B., Sedat, J. W., Alberts, B., and Agard, D. A. Microtubule nucleation by γ-tubulin-containing rings in the centrosome. Nature (Lond.), *378:* 638–640, 1995.

32. Kuriyama, R., and Borisy, G. G. Microtubule-nucleating activity of centrosomes in Chinese hamster ovary cells is independent of the centriole cycle but coupled to the mitotic cycle. J. Cell Biol., *91:* 822–826, 1981.

33. Burgess, S. A., Walker, M. L., Sakakibara, H., Knight, P. J., and Oiwa, K. Dynein structure and power stroke. Nature (Lond.), *421:* 715–718, 2003.

34. Steuer, E. R., Wordeman, L., Schroer, T. A., and Sheetz, M. P. Localization of cytoplasmic dynein to mitotic spindles and kinetochores. Nature (Lond.), *345:* 266–268, 1990.

35. Scholey, J. M., Porter, M. E., Grissom, P. M., and McIntosh, J. R. Identification of kinesin in sea urchin eggs, and evidence for its localization in the mitotic spindle. Nature (Lond.), *318:* 483–486, 1985.

36. Nigg, E. A., Blangy, A., and Lane, H. A. Dynamic changes in nuclear architecture during mitosis: on the role of protein phosphorylation in spindle assembly and chromosome segregation. Exp. Cell Res., *229:* 174–180, 1996.

37. Dutertre, S., Descamps, S., and Prigent, C. On the role of aurora-A in centrosome function. Oncogene, *21:* 6175–6183, 2002.

38. Giet, R., Uzbekov, R., Cubizolles, F., Le Guellec, K., and Prigent, C. The *Xenopus laevis* aurora-related protein kinase pEg2 associates with and phosphorylates the kinesin-related protein XlEg5. J. Biol. Chem., *274:* 15005–15013, 1999.

39. Hodgman, R., Tay, J., Mendez, R., and Richter, J. D. CPEB phosphorylation and cytoplasmic polyadenylation are catalyzed by the kinase IAK1/Eg2 in maturing mouse oocytes. Development (Camb.), *128:* 2815–2822, 2001.

40. Groisman, I., Jung, M. Y., Sarkissian, M., Cao, Q., and Richter, J. D. Translational control of the embryonic cell cycle. Cell, *109:* 473–483, 2002.

41. Marumoto, T., Hirota, T., Morisaki, T., Kunitoku, N., Zhang, D., Ichikawa, Y., Sasayama, T., Kuninaka, S., Mimori, T., Tamaki, N., Kimura, M., Okano, Y., and Saya, H. Roles of aurora-A kinase in mitotic entry and $G_2$ checkpoint in mammalian cells. Genes Cells, *7:* 1173–1182, 2002.

42. Chen, S. S., Chang, P. C., Cheng, Y. W., Tang, F. M., and Lin, Y. S. Suppression of the STK15 oncogenic activity requires a transactivation-independent p53 function. EMBO J., *21:* 4491–4499, 2002.

43. Gigoux, V., L'Hoste, S., Raynaud, F., Camonis, J., and Garbay, C. Identification of Aurora kinases as RasGAP Src homology 3 domain-binding proteins. J. Biol. Chem., *277:* 23742–23746, 2002.

44. Kimura, M., Matsuda, Y., Yoshioka, T., Sumi, N., and Okano, Y. Identification and characterization of STK12/Aik2: a human gene related to aurora of *Drosophila* and yeast IPL1. Cytogenet. Cell Genet., *82:* 147–152, 1998.

45. Mahadevan, D., Bearss, D. J., and Vankayalapti, H. Structure-based design of anticancer agents targeting aurora kinases. Curr. Med. Chem., *3:* 25–34, 2003.

46. Cheetham, G. M., Knegtel, R. M., Coll, J. T., Renwick, S. B., Swenson, L., Weber, P., Lippke, J. A., and Austen, D. A. Crystal structure of aurora-2, an oncogenic serine/threonine kinase. J. Biol. Chem., *277:* 42419–42422, 2002.

47. Francisco, L., and Chan, C. S. Regulation of yeast chromosome segregation by Ipl1 protein kinase and type 1 protein phosphatase. Cell. Mol. Biol. Res., *40:* 207–213, 1994.

48. Reich, A., Yanai, A., Mesilaty-Gross, S., Chen-Moses, A., Wides, R., and Motro, B. Cloning, mapping, and expression of ial, a novel *Drosophila* member of the Ipl1/aurora mitotic control kinase family. DNA Cell Biol., *18:* 593–603, 1999.

49. Schumacher, J. M., Ashcroft, N., Donovan, P. J., and Golden, A. A highly conserved centrosomal kinase, AIR-1, is required for accurate cell cycle progression and segregation of developmental factors in *Caenorhabditis elegans* embryos. Development (Camb.), *125:* 4391–4402, 1998.

50. Schumacher, J. M., Golden, A., and Donovan, P. J. AIR-2: an Aurora/Ipl1-related protein kinase associated with chromosomes and midbody microtubules is required for polar body extrusion and cytokinesis in *Caenorhabditis elegans* embryos. J. Cell Biol., *143:* 1635–1646, 1998.

51. Woollard, A., and Hodgkin, J. Stu-7/air-2 is a *C. elegans* aurora homologue essential for chromosome segregation during embryonic and post-embryonic development. Mech. Dev., *82:* 95–108, 1999.

52. Terada, Y., Tatsuka, M., Suzuki, F., Yasuda, Y., Fujita, S., and Otsu, M. AIM-1: a mammalian midbody-associated protein required for cytokinesis. EMBO J., *17:* 667–676, 1998.

53. Shindo, M., Nakano, H., Kuroyanagi, H., Shirasawa, T., Mihara, M., Gilbert, D. J., Jenkins, N. A., Copeland, N. G., Yagita, H., and Okumura, K. cDNA cloning, expression, subcellular localization, and chromosomal assignment of mammalian aurora homologues, aurora-related kinase (ARK) 1 and 2. Biochem. Biophys. Res. Commun., *244:* 285–292, 1998.

54. Tanaka, T., Kimura, M., Matsunaga, K., Fukada, D., Mori, H., and Okano, Y. Centrosomal kinase AIK1 is overexpressed in invasive ductal carcinoma of the breast. Cancer Res., *59:* 2041–2044, 1999.

55. Sakakura, C., Hagiwara, A., Yasuoka, R., Fujita, Y., Nakanishi, M., Masuda, K., Shimomura, K., Nakamura, Y., Inazawa, J., Abe, T., and Yamagishi, H. Tumour-amplified kinase BTAK is amplified and overexpressed in gastric cancers with possible involvement in aneuploid formation. Br. J. Cancer, *84:* 824–831, 2001.

56. Sen, S., Zhou, H., Zhang, R. D., Yoon, D. S., Vakar-Lopez, F., Ito, S., Jiang, F., Johnston, D., Grossman, H. B., Ruifrok, A. C., Katz, R. L., Brinkley, W., and Czerniak, B. Amplification/overexpression of a mitotic kinase gene in human bladder cancer. J. Natl. Cancer Inst. (Bethesda), *94:* 1320–1329, 2002.

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