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Review Article

Aurora kinases: therapeutic potential

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Abstract

Aurora mitotic protein kinases (A, B and C) are frequently overexpressed in cells from various tumor types. Additionally, overexpression of Aurora-A is sufficient to transform NIH/3T3 cells to induce tumor formation when implanted in nude mice. Altogether, these results have led many pharmaceutical companies to design Aurora kinase inhibitors with the hope of discovering new anticancer molecules. Several novel compounds that target the ATP pocket have recently been discovered. In this review we suggest other alternative strategies that might be useful for specifically inhibiting individual Aurora kinases.

Introduction

Cell cycle

The cell cycle describes an ordered set of events involving four phases. Of these, the S-phase (DNA synthesis) is the phase during which the genome is replicated, and the M-phase (mitosis) is the phase during which nuclear division occurs (chromosome segregation). Mitosis itself is a five-stage process (prophase, prometaphase, metaphase, anaphase and telophase); at the end of mitosis, one cell gives rise to two daughter cells that have identical copies of chromosomes. During prophase, the chromatin condenses to form chromosomes made of two sister chromatids joined by their centromere region, while the nuclear envelope disassembles. Simultaneously, the centrosome that has been duplicated at the end of the S-phase separates and migrates around the nucleus to reach opposite sides. They serve as microtubule-organizing centers to assemble the bipolar mitotic spindle. In prometaphase, microtubules connect the chromosome kinetochores (protein structures localized at the centromere) to each centrosome. Chromosomes then assemble in the middle of the cell to form the metaphase plate prior to being equally divided into each daughter cell. A balance of forces keeps the chromosomes under tension throughout metaphase until they acquire a correct bipolar attachment at their kinetochores. During anaphase, the sister chromatids of each chromosome separate and are pulled toward opposite poles; this motion is generated by motor proteins and by a decrease in the length of microtubules. Simultaneously, the midzone is organized by a redistribution of different molecules such as actin and myosin, which will ultimately serve to form the cleavage furrow. Once chromatids have completed their migration, a new nuclear envelope reforms around each nucleus and chromatin starts to decondense during telophase. Finally, an actin contractile ring is formed which initiates the process of cytokinesis. The midzone, called the postmitotic bridge at this stage, is then dissolved and the cytoplasm membrane of each daughter cell restored before completion of cytokinesis.

Cell cycle regulation

To ensure that the two daughter cells receive identical copies of the genome, progression through the cell cycle is highly regulated and controlled by the sequential synthesis, activation and subsequent degradation of proteins, which sometimes require a series of post-translational modifications. Protein phosphorylation and dephosphorylation play an important role in the control of mitotic events, for instance. Among the most well-known major mitotic protein kinases are CDK1 (cyclin-dependent kinase 1), PLK (polo-like kinase), Bub1

(budding uninhibited by benzimidazole 1), Nek2 from the NIMA family (never in mitosis A) and the Aurora kinases. All these kinases are implicated in various mitotic events such as G_2/M transition, checkpoints and cytokinesis (1).

Aurora kinases have attracted much attention since they have been found to be overexpressed in many cancers and since Aurora-A has been classified as an oncogene. Consequently, the Aurora kinases have been considered as priority drug targets in cancer therapy. In this review, we will first describe each member of the vertebrate Aurora kinase family and their involvement in cancer. Finally, we will focus on Aurora kinase inhibitors that are currently being developed for anticancer therapy and other possible molecules that could be developed to counteract the oncogenic activity of these kinases.

Aurora kinase family

The Aurora kinase family of serine/threonine kinases is composed of three members –A, B and C– in vertebrate species, which are key regulators of different mitotic events (2). The subcellular localization and function differ within the family and the protein kinase activity of each member is cell cycle-dependent. Indeed, their activities begin to gradually increase at the S-phase to peak at the M-phase in parallel with elevation of their mRNA and protein levels. Subsequently, the kinases are degraded by the proteasome upon exit from mitosis. Overexpression of any of the Aurora kinases (active or inactive) leads to an increase in ploidy, mainly due to centrosome amplification and/or misregulation of mitotic checkpoints.

Aurora-A

Aurora-A localizes to the centrosomes and is also found associated with microtubules at the spindle poles during mitosis (3). The kinase activity is involved in centrosome separation and maturation, as well as in bipolar spindle assembly and stability. Thus, its overexpression or inhibition leads to several spindle defects (4). Furthermore, it has been reported that its ectopic expression in immortalized NIH/3T3 cell lines is sufficient to provoke their transformation, defining Aurora-A kinase as an oncogene (5, 6). Exclusively the active kinase would be oncogenic; kinase-dead mutants of Aurora-A do not induce cell transformation. The oncogenicity of Aurora-A was questioned by a study that showed no transformation of primary cells when Aurora-A was overexpressed. We concluded from these studies that a background of genetic mutation is necessary for Aurora-A overexpression to transform cells, facilitating colony formation *in vitro* and tumor growth *in vivo* (7). This was recently confirmed in transgenic mice overexpressing the kinase in mammary cells using an inducible system. Zhang *et al*. reported that the mice did not develop malignant tumors (8).

Aurora-B

Aurora-B is a chromosomal passenger protein that is enriched on the chromosome kinetochores from prophase to metaphase, in the midzone during anaphase and in postmitotic bridges during telophase. It is responsible for the phosphorylation of histone H3 on both Ser10 and Ser28 and of centromere protein A (CENP-A) on Ser7 during the early metaphase (9, 10). The function of these modifications has not been clearly elucidated to date, but they seem to be required for the maintenance of proper chromosome dynamics during mitosis, presumably by helping protein loading on chromosomes. Aurora-B also regulates the kinetochore attachment to microtubules during metaphase, controlling that the cell exits metaphase only when all the chromosomes have acquired a bipolar attachment to microtubules arising from each centrosome (amphitelic attachment). Indeed, Aurora-B can prevent syntelic or merotelic attachments, which keep the checkpoint off despite the fact that the attachments are incorrect. In those cases (syntelic or merotelic), the kinase induces a monotelic attachment during which the checkpoint is activated, awaiting a correct amphitelic attachment of the chromosomes (11) (Fig. 1). Aurora-B localization at the midbody is also consistent with a role in the later stages of mitosis. Aurora-B plays an important role during cytokinesis (12, 13) by activating Rho kinases such as MLCK (myosin II light chain kinase), which regulates myosin by a direct or indirect pathway (14). Myosin is involved in the contraction of the actin cleavage furrow during cytokinesis.

Aurora-C

The last member of the Aurora family has not yet been well characterized. Although the protein has been found to be overexpressed in some tumor cells, the kinase may only be involved in spermatogenesis (15). First reported as a centrosome protein only from anaphase to cytokinesis (15), it has recently been described as a chromosome passenger protein (16, 17). Its function is presently unclear since no substrate or partner has yet been identified.

Aurora kinases and cancer

Aneuploidy, an abnormality in gene copy number, is the most prevalent cell genomic alteration identified in human solid tumors (18). Centrosome defects (number, organization and behavior) have also been found in many cancerous cell types, and could affect normal segregation of chromosomes and produce aneuploid cells (19, 20). A defect in centrosome maturation has been described in breast, cervical and prostate carcinomas (21). Colorectal tumors exhibit a defect in chromosome segregation leading to aneuploid cancerous cells (22). Centrosome amplifications inducing multipolar mitotic spindles have also

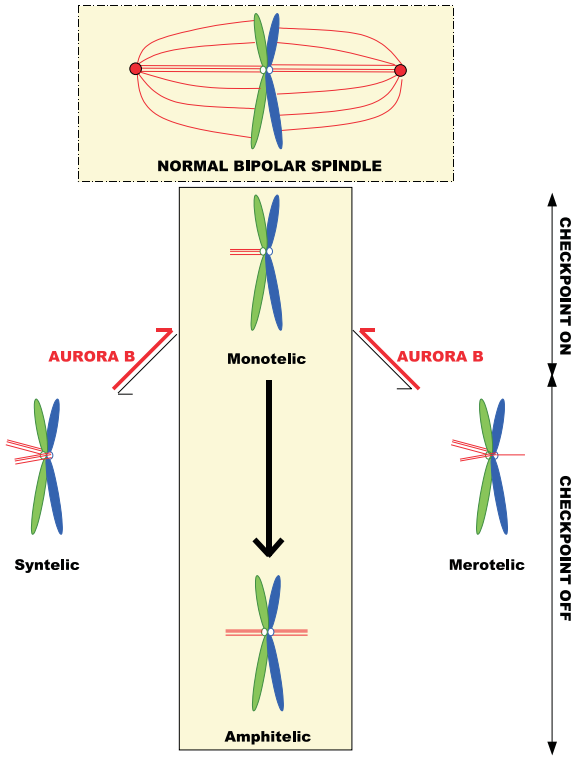

Fig. 1. Aurora-B is involved in correcting kinetochore-microtubule attachment. A syntelic attachment occurs when both kinetochores join to the same spindle pole. If one kinetochore attaches to both spindle poles, attachment is merotelic. Amphitelic attachment is the correct one, giving biorientation to the chromosome. Monotelic attachment (one kinetochore bound, one kinetochore free) triggers checkpoint activation, whereas syntelic, merotelic and amphitelic attachments allow cell cycle progression. Therefore, Aurora-B is a key regulator of the equitable division of genetic information.

been reported in head and neck cancers (23). Moreover, numerical and structural centrosome abnormalities have been described in various lymphomas, and seem to correlate with the cancer grade (24, 25). All these defects contribute to the acquisition of a genetic instability typically seen in cancerous cells, as predicted by Boveri in 1914, who already theorized that tumors may become malignant as the result of abnormal chromosome numbers (26).

These two features, aneuploidy and centrosome abnormalities, are also detected after Aurora overexpression or inhibition, identifying these kinases as potential players contributing to cancer progression. Furthermore, overexpression of each Aurora kinase has been observed in many human cancer cells and studies carried out in various cancerous tissues have shown that chromosomal abnormalities are often detected in regions containing genes encoding Aurora kinases (Table I). For instance, 20q13 gene amplifications were first described using comparative genomic hybridization in breast, colorectal, bladder and ovarian cancers (27-30). In 1997, one of the genes in the 20q13 amplicon was originally named BTAK, for breast tumor amplified kinase (31), and later renamed Aurora-A kinase (2). Kinase gene amplification and kinase protein overexpression have now been well characterized in various tumor cells, including colorectal cancers (5, 12, 32), breast cancers (6, 33), ovarian cancers (34, 35), pancreatic cancers (36) and hepatocellular carcinomas (37). However, Aurora-A overexpression is not only attributed to gene amplification, but can also result from dysregulation of transcription or translation (5, 37).

To detect amplification of Aurora kinase genes, several techniques can be used, including Southern blot or FISH (fluorescence *in situ* hybridization). Similarly, mRNA and protein expression levels can be measured by Northern blot and Western blot, respectively. Aurora kinase activity in tumor tissues can be estimated using several methods. The Aurora kinases can be immunoprecipitated from tissue lysates with specific antibodies that do not block the kinase catalytic activity (38). *In vitro* protein kinase assays are then performed in the presence of [^{32}P]-ATP and immunoprecipated proteins using MBP or histone H3 as substrates. Aurora kinase activity is quantified by measurement of incorporated radioactivity (35, 36). To avoid the use of radioactivity, Aurora activity can be estimated by Western blot, detecting the phosphorylation of histone H3 on Ser10 using an antibody directed against the phosphorylated Ser10 (39). Finally, one can assess Aurora-A activity by Western blot using an antibody directed against the phosphorylated Thr288 located in the activation loop of Aurora-A; Thr288 is phosphorylated when the kinase is active (40).

To date, Aurora-A kinase is the only member of the Aurora family found to possess true oncogenic potential when overexpressed in cells carrying a genetic background of defects, which remain to be identified (5). Although Aurora-B does not transform any cell line, cells overexpressing Aurora-B induced the formation of aggressive tumors when implanted in nude mice (32). Whether Aurora-C shows such activity remains to be determined. Nevertheless, because Aurora kinases are involved in normal mitosis, the question arises as to whether it is a good idea to develop Aurora inhibitors for anticancer therapy. Because Aurora kinases (at least A and B) are necessary for cell division, delivering Aurora inhibitors in the whole body will kill all dividing cells and eventually the patient. As for the majority of anticancer drugs, Aurora inhibitors will have to be locally delivered. However, the fact that in adult organs most of the cells have differentiated and therefore do not proliferate might help.

Novel anticancer drugs inhibiting Aurora kinases

Only three Aurora kinase inhibitors have been published to date. These small-molecule inhibitors are hesperadin (41), ZM-447439 (42) and VX-680 (43, 44) and were designed from the Aurora-A crystal structure (45).

Table I: Overexpression of Aurora kinases has been found in many cancer cell lines and also in various tumor tissues. The data indicate the percentage of cell lines or tumors which overexpress kinases.

Cell lines/human tumors	Overexpression/amplification	References
Aurora-A		
Breast cancer cell lines	30-40%	6, 31, 55, 56
Ductal invasive carcinomas	94%	33, 57
Primary invasive breast tumors	29%	27
Node-negative breast carcinomas	15%	58
Primary breast carcinomas	15%	6, 3, 55, 59
Primary colorectal cancers	> 50%	5, 28, 60
Ovarian cancer cell lines	37.5%	6, 34
Sporadic ovarian tumors	44-54%	34, 35
Hereditary ovarian tumors	100%	34
Hepatic cancer cell lines	100%	37
Hepatocellular carcinomas	61%	37
Pancreatic carcinoma cell lines	100%	36
Pancreatic tumors	58%	36
Aurora-B		
Colorectal tumor cell lines	ND	12
Primary human colorectal cancers	ND	61
Aurora-C		
Breast cancer cell lines	ND	15
Hepatocellular carcinoma cell lines	ND	15
Primary human colorectal cancers	51.3%	60

Two other compounds have been briefly reported: compound 677, described in an abstract from the 2004 annual meeting of the American Society of Clinical Oncology (ASCO) (46), and AZD-1152, developed by AstraZeneca. The chemical structures of all these drugs share few homologies with the ATP chemical structure (Fig. 2). Furthermore, they all target the catalytic domain of the kinases and occupy the ATP-binding pocket of the enzymes. In theory, these molecules should inhibit all members of the Aurora family because the catalytic domain of these kinases is highly conserved. T-loop activation has a unique conformation; as a consequence, these inhibitors are Aurora-specific and rarely interfere with other kinase activities (45). Although these antagonists are expected to inhibit all three Aurora kinases, this might not be a problem in terms of therapy. Indeed, although only Aurora-A is considered as an oncogene, it has been reported that cells overexpressing Aurora-B induce aggressive tumors when implanted in nude mice (32). Regarding Aurora-C, one might expect the same phenotype as Aurora-B (16). Moreover, the three kinases are often simultaneously overexpressed in cancer cells, and if only one of them is overexpressed, it might interfere with substrates and partners of the others.

VX-680

Vertex Pharmaceuticals developed VX-680, which has been shown to potently and selectively inhibit the Aurora kinases with apparent inhibition constants (K_{iapp}) of 0.6, 18 and 4.6 nM, respectively, for Aurora-A, -B and -C. However, VX-680 also appears to inhibit the FLT3 kinase (Fms-related tyrosine kinase-3), the gene which is often mutated in high-grade acute myelogenous leukemia (AML). This last kinase is also an interesting drug target.

Two studies using VX-680 have been conducted to explore its antitumor activity. The first showed that VX-680 caused cell cycle arrest in a variety of cancer cell lines; some stopped proliferating with a tetraploid DNA content (43). Apoptotic cell death usually followed the arrest, and leukemia, lymphoma and colorectal cancer cell lines were the most sensitive to the drug. VX-680 may therefore cause tumor regression. The effects on primary tumor samples from patients with AML who were refractory to standard treatment were then examined. Colony formation in these cells (including those in which FLT3 was mutated) was decreased by VX-680. The growth of human cancer (AML and colorectal) xenografts in nude animals was also decreased following treatment with VX-680. The inhibitor did not seem to affect noncycling cells and was able to prevent phosphorylation of histone H3, indicating that VX-680 specifically inhibits Aurora kinases. In another study, VX-680 was demonstrated to inhibit colony formation of primary leukemia cells (44). The authors confirmed that the growth of human AML tumor xenografts in nude mice was greatly reduced after VX-680 administration.

To date, VX-680 is the only Aurora kinase inhibitor reported to have therapeutic effects against cancer cells,

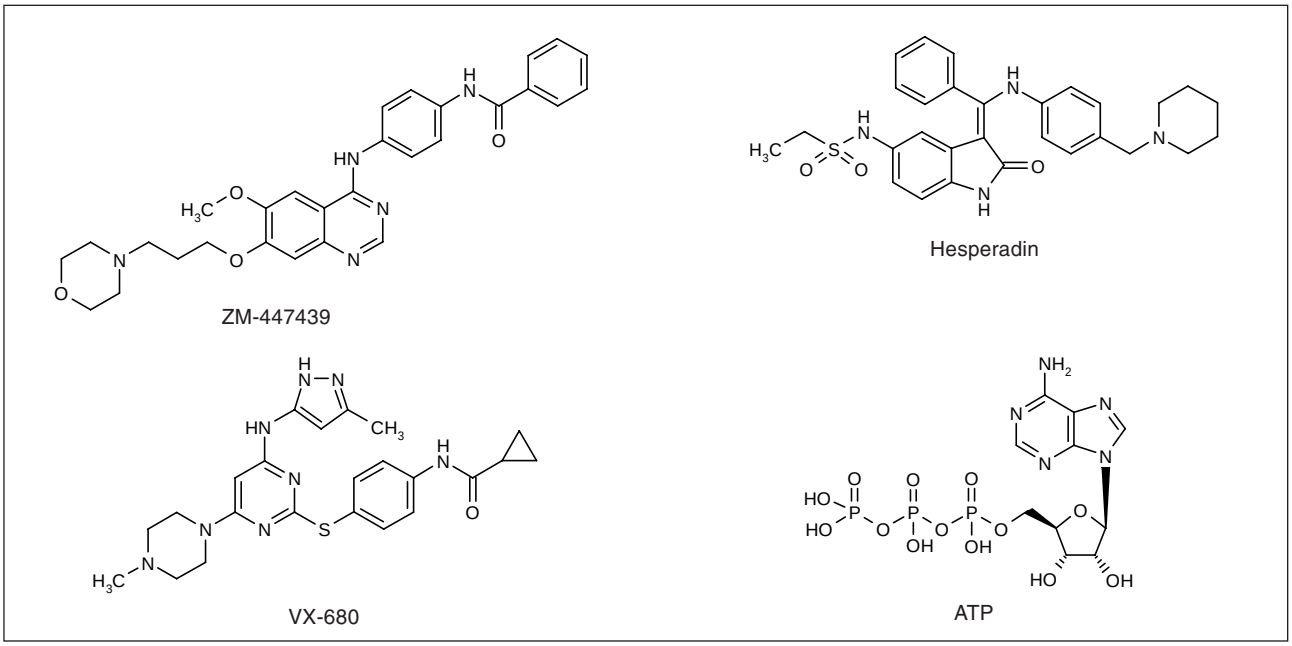

Fig. 2. Chemical structures of potent Aurora kinase inhibitors: ZM-447439 (42); hesperadin (41) and VX-680 (43). These compounds are ATP analogues and share 3-dimensional structural homology.

and it just recently progressed into clinical development (Merck & Co. purchased the license for this compound). The other Aurora inhibitors are mainly used at this time to dissect complex cellular processes in which Aurora kinase functions are involved. Their therapeutic effects on human cancer cells have not yet been demonstrated.

Hesperadin and ZM-447439

Both hesperadin (41) and ZM-447439 (42) have been used in fundamental research to study Aurora-B functions. The specificity of the inhibitor was first tested *in vitro*. Hesperadin is not a specific Aurora-B inhibitor since it also inhibits other protein kinases, including AMP-activated kinase (AMPK), Lck, mitogen-activated protein kinase (MAPK) kinase 1 (MKK1), MAPK-activated protein kinase 1 (MAPKAP-K1), the checkpoint kinase CHK1 and phosphorylase kinase (PHK). Surprisingly enough, hesperadin has apparently not been tested against Aurora-A. ZM-447439 has been shown to inhibit only Aurora-A and -B *in vitro*. The cell phenotype obtained after hesperadin or ZM-447439 administration resembled that obtained after Aurora-B knockdown by RNA interference (Fig. 3). For this reason, both inhibitors have been classified as Aurora-B inhibitors.

Using these new tools, Aurora-B functions have been explored in detail. For instance, it was shown that Aurora-B is required for histone H3 phosphorylation, for chromosome alignment (by regulating kinetochore attachment) and segregation (action on spindle checkpoint), for mid-spindle formation and for cytokinesis (41, 42). These Aurora inhibitors are also potential therapeutic agents as Aurora-B kinase has been shown to be overexpressed in some cancers (12, 32). Furthermore, results for ZM-447439 underlined the selective toxicity of this compound on proliferating tumor cells; while nondividing cells remain viable, cycling cells die. Noncycling cells do not express Aurora kinases during their quiescence.

Other inhibitors

As previously mentioned, compound 677 was only briefly described during the 2004 ASCO meeting (46). The research group evaluated its efficacy as a novel anticancer drug since it specifically inhibits Aurora-B. The HCT 116 colon cancer cell line was used as a research model to check polyploidy and apoptosis, and to observe spindle formation. Fewer defects were reported when cells were treated with this compound alone or in combination with chemotherapeutic agents. Thus, compound 677 appears to be an excellent inhibitor of Aurora-B and may be useful for stopping the growth of proliferating cancerous cells.

Similarly, AstraZeneca is developing an Aurora kinase inhibitor named AZD-1152, designed to target cell division in proliferating tumors. AZD-1152 is currently in preclinical development and little is known about this molecule.

All these inhibitors might be used either for their direct action against tumors or to modulate tumor sensitivity and resistance to other agents such as paclitaxel (7). To gain in specificity, it would also be interesting to

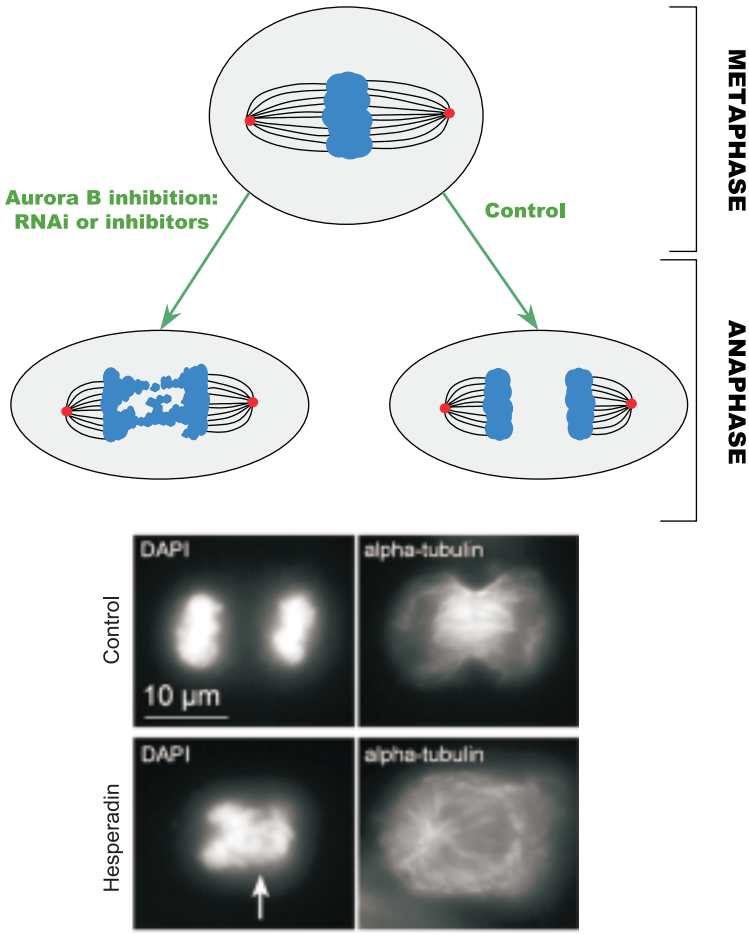

Fig. 3. Aurora-B is implicated in correct anaphase completion by checking the correct kinetochore-microtubule attachment. Treatment with ZM-447439 or hesperadin phenocopies Aurora-B RNAi, suggesting that they are potent and selective Aurora-B inhibitors. Indeed, HeLa cells treated for 16 h with hesperadin show a defect in chromatin segregation in anaphase (indicated by arrow). DNA was stained with DAPI and mitotic spindle with α-tubulin antibodies (41). (Images kindly provided by Jan Michael Peters).

develop new inhibitors that do not target the ATP binding site of Aurora kinases. In this sense, Aurora kinases possess an *N*-terminal domain which shares low sequence identity and serves for selective protein interaction and localization.

Other potential approaches

Aurora kinase activation inhibitors

Aurora kinases are activated *in vivo* by a unique mechanism that might be used as a specific drug target. Although the recombinant proteins show detectable kinase activity *in vitro* when produced in bacteria (especially Aurora-A), they are activated *in vivo* by autophosphorylation, mainly when the kinase meets its substrate. Interestingly, the substrates/activating proteins are different for Aurora-A and Aurora-B. This mechanism might represent a good opportunity to design drugs that will specifically target only one Aurora kinase. Aurora-A is activated by TPX2, or targeting protein for Xklp2 (*Xenopus* kinesin-like protein 2); the crystal structure of an Aurora-A/TPX2 complex has been resolved, giving a good indication of the 3-dimensional structural mechanism of the activation (47). Another protein called Ajuba has also been described as an Aurora-A activator (48), but the crystal structure of the complex is not available. In the case of Aurora-B, and this becomes interesting, inner centromere protein (INCENP) has been described as an activator (49) since it enables Aurora-B autophosphorylation (50). Survivin is another Aurora-B activator that binds its catalytic domain and also modulates its kinase activity (51). Both of these Aurora-B activators are unable to activate Aurora-A. Although Aurora-C activators remain to be found, it can be predicted that this kinase is activated by the same type of mechanism. A molecule that would interfere with the binding of the activator may provide a good way to inhibit the kinase.

Targeting the gene promoter

Aurora kinases are transcribed in a cell cycle-dependent manner and many cancers are characterized by overexpression of these kinases. In the case of Aurora-A, for instance, kinase overexpression has been reported in the absence of the 20q13 amplicon, indicating that other mechanisms may account for the increase in activity. Another anticancer therapeutic strategy could be used to decrease Aurora kinase transcription by targeting transcription factors that bind Aurora gene promoters. Two recent reports have described attempts to dissect the transcription regulation of Aurora kinase genes. In these studies, the 5'-flanking regions of Aurora-A and -B were sequenced. The Aurora-A promoter gene possesses a positive regulatory element (PRE) and a repressor sequence called cell cycle-dependent element-cell cycle homology region (CDE-CHR) (52), while the Aurora-B promoter contains at least the CDE-CHR sequence (53). The transcription factor E4TF1 positively regulates Aurora-A expression by interacting with the PRE box. This activation is cell cycle-independent because E4TF1 is a ubiquitous protein of the ETS family whose members regulate more than 200 genes; as a consequence, it is not a valuable target for therapy. On the other hand, a cell cycle-dependent mechanism regulates Aurora kinase transcription, as well as the transcription of several G_2/M-specific genes (CDC25C, cyclin A, cyclin B2, CDK1); this process involves the tandem repressor CDE-CHR. Different protein complexes that bind to CDE-CHR have been described, but this field remains controversial. The main complex identified to bind the CDE sequence belongs to the E2F/DP family. Indeed, E2F1, E2F4 and DP2 were reported to bind the CDE sequence of the Aurora-B gene promoter (53). These E2F/DP heterodimers are negatively regulated by retinoblastoma protein (Rb), which is itself inactivated by hyperphosphorylation at the end of mitosis. Thus, Aurora-B transcription does not occur during interphase and starts in the early G_2-phase when Rb is dephosphorylated (54). Depending on the gene, different complexes are implicated in transcription regulation, but some complexes can activate different promoter genes. We need to find the specific transcription factor for the gene. A better understanding of this complex regulation would be necessary in order to find the specific transcription factor for the Aurora genes, which may provide an ideal target.

Conclusions

The important role of the Aurora kinase family in cancer progression strongly indicates that they represent excellent targets for cancer treatment. However, the efficacy of the novel drugs described here needs to be assessed in cancer models. Other potential approaches may represent useful strategies to downregulate the expression of these kinases. Even if these approaches result in inefficient knockdown of Aurora expression, it should not be forgotten that extinction of its expression could induce other defects, since its activity is required for physiological events. Too good an inhibitor of Aurora kinase may be a bad chemotherapeutic drug.

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