

The role of Aurora-A inhibitors in cancer therapy

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Recently, new chemotherapy agents which target the non-structural components of mitosis have been developed. An important protein involved in several mitotic phases is the Aurora-A protein. By means of the phosphorylation of different substrates, Aurora-A regulates the correct development of the various phases of mitosis. The kinase activity of this protein makes Aurora-A an excellent candidate as an oncogene. The first data of Aurora-A involvement in cancer regarded the identification of Aurora-A overexpression in primary breast and colon tumour samples. With regard to the predictive role of Aurora-A, it has been shown that its overexpression disrupts the spindle checkpoint activated by paclitaxel (Taxol) or nocodazole treatment, thus inducing the cells to become resistant to these drugs. The development therefore of small molecules with an Aurora-A inhibition function may make it possible to reduce or block the oncogenic activity of Aurora-A and in addition may improve the survival of oncological patients showing resistance to paclitaxel or nocodazole treatment. Three novel Aurora kinase inhibitors have recently been described—Hesperadin, ZM447439 and VX-680. All these three drugs have been designed to target the ATP-binding site of Aurora kinase, so they inhibit all three Aurora kinase family members showing a similar phenotype when tested in cell-based assays. Among these three different molecules, VX-680 has shown promising results in *in vitro* and *in vivo* studies. In conclusion, it is clear that we are entering a new era in anti-mitotic therapy with the identification and now clinical translation of new targets in mitosis beyond tubulin but many questions remain with regard to Aurora function.

Key words: Aurora-A, cancer treatment, kinase inhibitor, mitosis, small molecule

introduction

The process of cell division, mitosis, is a multiphase mechanism by which the duplicate DNA is completely segregated into two daughter cells. The main effectors of this division are the mitotic spindle and the centrosomes, two structures made up of microtubules which cooperate in the process of chromosome condensation, alignment on the metaphase plate and segregation. In a tumoural cell, aberrations of these processes generate aneuploid daughter cells with genomic instability and make it possible for apoptosis to occur. For this reason, several chemotherapy agents, such as vinca alkaloids and taxanes, have been developed and are currently used in clinical cancer treatment. The principal target of these agents is the tubulin, the main protein present in the mitotic spindle and in the centrosomes. These drugs usually induce stabilisation or destabilisation of the mitotic spindle structure and block the deregulated mitotic process of the tumoural cells.

Recently, new chemotherapy agents which target the non-structural components of mitosis have been developed. Each

phase of mitosis is, in fact, regulated by the presence of several mitotic kinesins and protein kinases that synchronise all phases and 'produce' tumoural cells. For instance, the mitotic kinesin spindle protein (KSP) is important for the regulation of mitotic spindle bipolarity by conducting centrosome separation and the use of KSP inhibitors induces apoptosis in some tumour cell lines [1, 2]. The centromeric protein E is involved in chromosome congression at the metaphase and its inhibitors are now in preclinical development [3]. Finally, Polo-like kinase 1 is required for centrosome maturation and the formation of the mitotic spindle and the use of small molecules or small interfering RNA have shown interference in several mitosis stages [4, 5].

the Aurora-A

Another important protein involved in several mitotic phases is the Aurora-A protein also known as serine threonine kinase 15 (STK15), BTAK, Aurora kinase A, Aurora-2 or AIKI. This gene is a member of the Aurora kinase family made up of Aurora-A, B and C and is conserved throughout eukaryotic evolution. Human Aurora-A is located at chromosome 20q13.2, which is commonly amplified in several tumoural tissues [6–13]. The protein structure of the Aurora-A is based on a variable amino terminal regulatory domain, with three

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putative Aurora boxes (A-box I, II and III), and a conserved carboxyl terminal catalytic domain, with an activation motif and a destruction box [14]. The functional significance of the three A-boxes is not fully understood but there is some evidence that they may be related to subcellular localisation or substrate recognitions. Aurora-A is a serine–threonine kinase which requires phosphorylation in order to become activated. The presence of three phosphoric groups, respectively, at residues serine 51, threonine 288 and serine 342 of the activation motif is particularly important for the function of this kinase. The serine 51 residue is involved in controlling the timing of Aurora-A destruction process during mitotic exit while the threonine 288 is relevant for the kinase activity. The T288D mutation, in fact, which mimics a constitutive phosphorylation status, gives rise to the presence of a constitutively active Aurora kinase. Finally, the serine 342 residue is principally involved in the maintenance of its conformational three-dimensional structure [14].

The levels of Aurora-A are usually regulated during the cell cycle phases by means of two different processes, ubiquitin-dependent proteolysis [15, 16] and phosphorylation/dephosphorylation events [17]. Aurora-A, in fact, is ubiquitinated by the anaphase-promoting complex/cyclosome (APC^{CDH1}) through the interaction with the destruction box (D-box) and the serine 51 residue of the A-box II. Moreover, a novel protein known as Aurora-A kinase-interacting protein seems to regulate the Aurora-A degradation through the proteasome-dependent pathway [18]. The mechanism of feedback regulation through phosphorylation/

dephosphorylation events during the cell cycle is, instead, mediated by the action of kinase and protein phosphatase 1 [19].

the Aurora-A protein in mitotic events

During the late-G₂ to M phase, the Aurora-A levels and kinase activity increase in order to be able to perform the role of ‘guardian of the poles’. By means of the phosphorylation of different substrates, in fact, Aurora-A regulates the correct development of the various phases of mitosis, including centrosome maturation and separation, mitotic entry, bipolar spindle assembly, chromosome alignment on the metaphase plate and cytokinesis [20] (Figure 1). In human cell lines, Aurora-A depletion results in the inhibition of both centrosome maturation [21] and centriole pairs’ separation [22]. The regulation of these two phases of mitosis occurs by means of the phosphorylation, respectively, of the transforming acidic coiled-coil (TACC) proteins, which cooperate in the growth of the microtubules at both the minus and the plus ends, and of the kinesin-like motors, needed for centrosome separation during the prometaphase.

The commitment of cells to mitosis in the late G₂ phase involves the activation of both Aurora-A and CDK1–cyclin B. This activation is a kind of feedback mechanism in which the Aurora-A activation requires the CDK1–cyclin B activation [23] and the CDK1–cyclin B complex is activated by the Aurora-A-dependent phosphorylation of CDC25B at serine

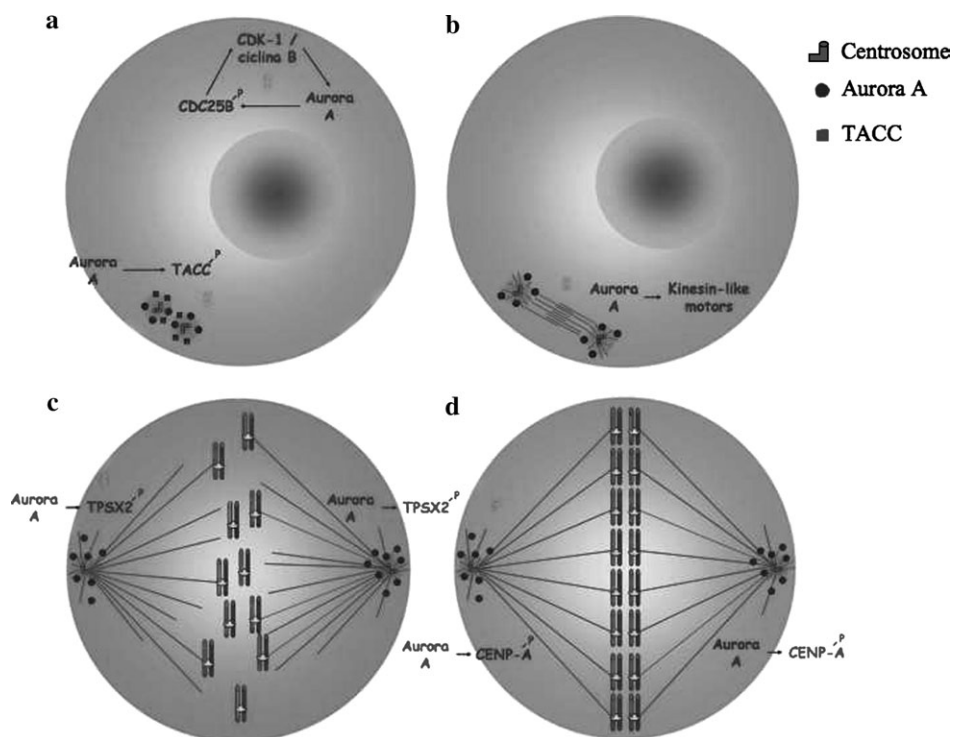


Figure 1. (A) Aurora-A is first detected in late G₂ phase at the centrosome, where it is involved in the commitment to mitosis (1) and in maturation of centrosome through the phosphorylation of TACC (2). (B) Aurora-A is required for the separation of centrosomes in prometaphase through the activation of the kinesin-like motors (3). (C) For the bipolar spindle assembly (4). (D) Aurora-A cooperates with Aurora-B in the activation and maintenance of CENP-A (5), allowing a correct chromosome alignment on the metaphase plate.

353, the phosphatase involved in cyclin-dependent activation [21].

Aurora-A protein regulates bipolar spindle assembly by means of the interaction with the TPX2 protein, which is both a substrate and an activator of Aurora-A. Experiments conducted singly on Aurora-A and TPX2 depletion, in fact, have shown in both these the formation of multipolar spindles [20, 24].

Several data reported in literature have shown that the chromosome alignment on the metaphase plate is principally regulated by the Aurora-B protein [25–27]. Recent studies [28] have shown that both Aurora-A and Aurora-B are involved in this step by means of the phosphorylation at the serine 7 residue of the CENP-A, the protein responsible for the interaction between microtubules and the kinetochore. More specifically, Aurora-A is responsible for the first CENP-A phosphorylation and activation while Aurora-B is responsible for maintaining this activation status from the late prophase to the metaphase.

A perfect timing of Aurora-A activation and destruction is necessary for a proper cytokinesis. Both Aurora-A overexpression and inhibition, in fact, lead to multinucleated cell formation [29–33]. Finally, since the dephosphorylation of several Aurora-A substrates occurs, as previously mentioned, through Aurora-A degradation in an APC^{CDH1}-dependent manner, this represents a key event in the completion of cytokinesis [16].

Aurora-A and cancer

The first data of Aurora-A involvement in cancer regarded the identification of Aurora-A overexpression in primary breast and colon tumour samples [34, 35]. Subsequently, several other studies showed chromosome 20q13 amplification or Aurora-A overexpression in many different tumour types including breast, pancreatic, ovarian and gastric cancers [6–13]. Furthermore, conflicting data have been reported regarding Aurora-A overexpression and tumour grade. In fact, whereas some authors suggest that Aurora-A overexpression is an early pathological event in cancer progression both in animal models [36] and in patients affected by ovarian cancer [11], others indicate a significant association with higher grade tumours and poor prognosis [37].

Mitosis is an extremely important biological event regulated by Aurora-A, and this fact together with the kinase activity of this protein makes Aurora-A an excellent candidate as an oncogene. Several studies have, in fact, reported a specific role of Aurora-A in cancer development and progression. For instance, unlike normal cells, in tumour cells Aurora-A expression may be detected diffusely in all the cytoplasm [11], giving rise to aberrant phosphorylated cytoplasmic proteins. A large number of studies taking into consideration the kinase activity of the protein have reported a relationship between Aurora-A, the p53 tumour suppressor protein and cancer progression [38–40]. Aurora-A, in fact, phosphorylates p53 at the serine 215 residue, inducing an inactivation of transactivation activity, and at the serine 315 residue, facilitating MDM2-mediated p53 degradation. Moreover, an association has been shown between Aurora-A overexpression,

p53 mutational status and a worse prognosis in patients affected by hepatocellular carcinomas [37]. Finally, cells with Aurora-A overexpression and with a non-functional post-mitotic G₁ checkpoint are usually tetraploid [29, 30].

With regard to the predictive role of Aurora-A, it has been shown that its overexpression disrupts the spindle checkpoint activated by paclitaxel (Taxol) or nocodazole treatment, thus inducing the cells to become resistant to these drugs [30, 41]. The development therefore of small molecules with an Aurora-A inhibition function may make it possible to reduce or block the oncogenic activity of Aurora-A and in addition may improve the survival of oncological patients showing resistance to paclitaxel or nocodazole treatment.

Aurora-A kinase inhibitors

In the last few years, several drugs with an inhibition action towards Aurora-A kinase have been developed and several protein kinase inhibitors, already present in other preclinical studies, have been tested to assess the specificity to Aurora-A. The identification of Aurora-A inhibitors may require the choice of two different approaches—blocking the protein–protein interaction between Aurora-A and cofactor or substrates or blocking the ATP-binding site of the serine threonine kinase. Most of the small molecules with Aurora-A inhibitor function identified so far show good specificity for the ATP-binding site but less for the target protein and this may be a problem if all the protein kinases involved in mitosis are taken into consideration. This difficulty increases in the presence of an Aurora kinase family where the action of each member during mitosis may be overlapped and is not yet fully understood. Even if Aurora-A is considered the principal member of the family with oncogenic activity, therefore, it may well be that the anticancer action of some chemotherapy agents do not reflect the blocking activity on Aurora-A but on other family members (mainly Aurora-B) or protein kinase. For this reason, we will refer to these small molecules as Aurora kinase family inhibitors instead of specifying their role towards the Aurora-A protein. An explanation of the effects of the inhibition action of these small molecules is now possible. In the presence, in fact, of an anti-Aurora compound, which inhibits both Aurora-A and Aurora-B, the abnormal spindles arising from the lack of Aurora-A activity might not be visible because the absence of Aurora-B activity will not trigger mitotic arrest and cytokinesis. Thus the only phenotype that will be observed will look similar to the one observed after Aurora-B down-regulation. Table 1 shows the main Aurora kinase inhibitors which have already undergone preclinical or phase I/II clinical trials. Unfortunately, very few data are available since a great many of these molecules are in still in the experimental phase of each study.

Aurora kinase inhibitors in preclinical studies

The JNJ-7706621 molecule is a [1,2,4]triazole-3,5-diamine with a dual inhibitor effect on cyclin-dependent kinases and Aurora kinase family members but with a unique inhibitor profile [42].

Table 1. Main Aurora kinase inhibitors which have already undergone preclinical or phase I/II clinical trials

Preclinical	Phase I	Phase II
CHR-3520	PHA-739358	VX-680
CTK-110	AT-9283	
CYC-116	MLN-8054	
ENMD-981693	R-763	
JNJ-7706621	SU6668	
PHA-680632	Hesperadin	
SNS-314	ZM447439	
MP-529		
MP-235		

In more specific terms, the treatment of human cancer cells with this molecule induces a cell growth inhibition independent of p53, pRB and P-glycoprotein status; apoptosis activation and the reduction of colony formation while in human tumour xenograft models several dosing schedules with an antitumoural activity have been identified. Furthermore, it has been shown that JNJ-7706621 treatment at low concentrations induces a slowing down of cell growth, while at high concentrations, it leads to cytotoxicity. With regard to the JNJ-7706621 effect on the Aurora kinase family member, preliminary studies have shown that the treatment of cells with this molecule induces endoreduplication and loss of histone H3 phosphorylation, all consequences of Aurora kinase and particularly of Aurora-B inhibition.

The PHA-680632 molecule, a novel Aurora kinase inhibitor, shows an anticancer activity on a wide range of different cancer cell lines and on different animal tumour models at well-tolerated doses. In this case, too, the effect of this anticancer activity on cancer cells is reported as the inhibition of the histone H3 phosphorylation at the serine 10 residue.

Aurora kinase inhibitors in clinical studies

Unfortunately, most of the Aurora kinase inhibitors showing a positive response in preclinical studies and which at present are being analysed in clinical studies are in early-phase trials and no specific information is available at this time.

Until now, knowledge regarding the mechanism of action of protein kinase inhibitors has mainly involved the final effect on mitosis and not on the different substrates. For this reason, several studies have been conducted in order to investigate whether or not selective kinase inhibitors involved in other biological events may have an effect on Aurora proteins. For example, experiments regarding chemical proteomics have shown that the molecule SU6668, originally designed as an inhibitor of receptor tyrosin kinases implicated in tumour vascularisation, also has the Aurora kinases as substrate [43]. The observation, therefore, that SU6668 treatment of HeLa cells leads to a reversible G₂-M block consistent with Aurora inactivation may make it possible to develop future strategies for more efficient therapeutic intervention in cancer based on

both receptor tyrosine kinase (RTKs), involved in tumour vascularisation, and Aurora kinases, involved in mitotic progression targeting.

Three novel Aurora kinase inhibitors have recently been described—Hesperadin [26], ZM447439 [25] and VX-680 [44]. Hesperadin in particular has a structure similar to the SU6668 molecule, ZM447439 is the first inhibitor to be characterised and VX-680 is the first Aurora kinase inhibitor to enter clinical trials. All these three drugs have been designed to target the ATP-binding site of Aurora kinase, so they inhibit all three Aurora kinase family members showing a similar phenotype when tested in cell-based assays. Among these three different molecules, VX-680 has shown promising results in *in vitro* and *in vivo* studies. *In vitro* VX-680, in fact, blocks cell proliferation, disrupts bipolar spindle formation, causes accumulation of cells with 4N or greater DNA and eventual cell death while *in vivo* it is able to cause regression in xenograft models of leukaemia and colon cancer (HL-60 and HCT-116) at well-tolerated doses [44]. Moreover, VX-680 has no effect on non-cycling primary human cells, probably because expression and activity of Aurora kinases is low in normal cells and this makes this molecule a promising anticancer drug. Finally, various protracted intravenous infusions have been tested in phase I trials for patients with solid and haematological malignancies. The main dose-limiting toxicity in the solid tumour study was neutropoenia. Unlike other anti-mitotic agents such as taxanes, it seems that the main dose-limiting toxicity for VX-680 is neutropoenia and no significant clinical neuropathy has been observed.

A study of the crystal structure of VX-680 bound to Aurora-A shows the potency and selectivity profile of this molecule. Cheetam Harrington and co-workers have, in fact, demonstrated that VX-680 weakly binds Aurora-A open active conformation and subsequently traps the enzyme in a closed inactive conformation by binding and stabilising the hydrophobic activation loop [44]. The high degree of selectivity of VX-680, therefore, depends on the fact the Aurora kinases are able to adopt a closed conformation while other protein kinases are not.

The effect of longer exposure to these three Aurora kinase inhibitors is different according to the p53-dependent post-mitotic checkpoint status [25, 45]. P53, in fact, is able to respond to a failed cell division inducing a G₁-like arrest as a sort of back up to the spindle checkpoint arrest. Thus, the cell lines with a wild-type or active p53 underwent apoptosis or arrest in a pseudo-G₁ phase while the cell lines with a mutated or inactive p53 became polyploid.

In contrast to Aurora-B inhibition, Aurora-A inhibition causes a delay and not a block in mitotic entry and a chromosome alignment that is found in aneuploid cells [22]. In this case, the Aurora-A inhibitors may have an ‘oncogenic effect’ generating chromosome instability. Thus, even though the Aurora kinase inhibition brought about by these different drugs has shown primary results in cancer treatment, it might well be that the chromosome instability induced by them will generate aberrant mitotic events that promote tumorigenesis. The mechanisms and signalling cascades by which Aurora-A inhibitors induce cell death must therefore be clarified.

conclusions

The principal goal in the development of Aurora kinase inhibitors is to assess whether or not the administration of these small molecules to patients will yield a clinical benefit. For this reason it is essential to answer several different questions such as those regarding the effect of these inhibitors on other kinase proteins, the effect of the same drugs on the three different members of the Aurora kinase family and the pathways and the protein involved in Aurora kinase inhibition. For example, the interaction between Aurora kinase and p53 might indicate a patient's inclusion in the study according to the p53 status. Furthermore, it will be important to identify the safe dose for target inhibition in humans, the tumour types that most likely respond to these drugs, the reversibility of the effect on normal cells and the dependence of this on dose and duration of exposure, the toxicity effects observed in patients, the effect of these drugs on disease-free survival and overall survival and the effect of these drugs when used in combination with other chemotherapy agents, in particular, those which depend on the spindle checkpoint such as taxanes and others.

In conclusion, it is clear that we are entering a new era in anti-mitotic therapy with the identification and now clinical translation of new targets in mitosis beyond tubulin but many questions remain with regard to Aurora function and the answers will be of great interest, not only to basic researchers but also to clinicians and patients.

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