Chapter 4

# Aurora Kinases: New Molecular Targets for the Therapy of Aggressive Thyroid Cancers

Enke Baldini, Chiara Tuccilli, Salvatore Sorrenti, Domenico Mascagni, Stefano Arcieri, Angelo Filippini and Salvatore Ulisse

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**Abstract**

Epithelial thyroid carcinomas (TC) account for more than 90% of all endocrine malignancies and represent one of the most frequent cancers in women. They include the well-differentiated TC (DTC), comprising the papillary (PTC) and follicular (FTC) histotypes, the poorly differentiated (PDTC), and the undifferentiated or anaplastic TC (ATC). Both PDTC and ATC are aggressive human neoplasms with a dire prognosis due to the absence of effective therapies, which makes mandatory the identification of novel therapeutic strategies. Intrinsic chromosomal instability (CIN, an increased rate of gain or losses of chromosomes during cell division) is a common feature of solid tumors and represents a major driving force in thyroid cancer progression, thought to be responsible for the acquisition by malignant cells of novel functional capabilities. Different mitotic kinases, whose expression or function has been found altered in human cancer tissues, are major drivers of thyroid tumor aneuploidy. Among these are the three members of the Aurora family (Aurora-A, Aurora-B and Aurora-C), serine/threonine kinases that regulate multiple aspects of chromosome segregation and cytokinesis. Over the last few years, several small molecule inhibitors targeting Aurora kinases were developed with promising antitumor effects in preclinical and clinical studies against different human cancers, including TC. Here, we will focus on the Aurora mitotic functions in normal cells; we shall then describe the main implications of their overexpression in the onset of genetic instability and aneuploidy. We will finally describe the consequences of Aurora kinase inhibition on TC cell growth and tumorigenicity.

**Keywords:** thyroid cancers, Aurora kinases, mitosis, therapy, Aurora kinase inhibitors

## 1. Introduction

The incidence of thyroid cancer (TC) has increased from about five new cases per 100,000 persons observed in the early 90s to 15 new cases per 100,000 persons recorded in 2012 [1]. This increase is mainly due to the improved ability to detect malignancy in small thyroid nodules [2, 3]. TC represents about 96% of all endocrine malignancies and one of the most frequent cancers in women [1]. Based on histological and clinical criteria, TC are classified as well-differentiated TC (DTC), which includes the papillary (PTC) and follicular (FTC) histotypes, poorly differentiated TC (PDTC), and anaplastic TC (ATC). The PTC accounts for about 86% of all epithelial TC. It appears as a mass of branching papillae covered by cells with eosinophilic cytoplasm and enlarged nuclei and typically metastasizes via lymphatic vessels to local lymph nodes [4]. The FTC accounts for approximately 9% of all TC. It resembles the normal microscopic pattern of the thyroid and is characterized by hematogenous spread producing lung and bone metastases [4]. The less differentiated and more aggressive PDTC and ATC, each of which accounts for 1–2% of all TC, are thought to develop from the dedifferentiation of DTC, according to the multistep model of thyroid carcinogenesis [4–8]. The PDTC was included as a separate entity in the WHO classification of TC in 2004. PDTC retains sufficient differentiation to produce scattered small follicular structures and some thyroglobulin but generally lacks the usual morphologic characteristics of DTC, showing an intermediate clinical behavior between DTC and ATC. In addition, it is characterized by high-grade features such as widely infiltrative growth, necrosis, vascular invasion, and numerous mitotic figures [6, 9]. The ATC is composed of disseminated fleshy masses with areas of necrosis and hemorrhage. The cells have an undifferentiated phenotype with marked cytological atypia and high mitotic activity, and they are negative for thyroglobulin [4].

Established risk factors for TC include radiation exposure, family history of TC, lymphocytic thyroiditis, reduced iodine intake, and female gender [10]. All of them are thought to induce chromosome instability (CIN) in thyrocytes through still poorly defined direct and indirect mechanisms [10–13]. Actually, number and frequency of chromosomal abnormalities increase from DTC to PDTC and ATC [13]. CIN is also sustained by alterations in cell cycle regulators, frequently encountered in TC [10]. In particular, a deregulated control of the G1/S transition, following either an increased expression of promoting factors (cyclin D1 and E2F) or the downregulation or presence of loss-of-function mutations of factors inhibiting the G1/S transition (retinoblastoma, p16INK4A, p21CIP1, p27KIP1, and p53), has been documented in TC [10]. In addition, the aberrant expression of mitotic kinases, such as the polo-like kinase and the three members of the Aurora kinase family, is held co-responsible for abnormal cell divisions and the establishment of aneuploid TC cells [14, 15].

About 80% of PTC are characterized by mutually exclusive activating somatic mutations of genes encoding for proteins involved in the mitogen-activated protein kinase (MAPK) signaling pathway [4, 16]. These include rearrangements of the *RET* (rearranged during transformation) (RET/PTC) and neurotrophic tyrosine kinase receptor 1 (*NTRK1*) genes, and activating point mutations of the three *RAS* oncogenes (*HRAS*, *KRAS*, and *NRAS*) and *BRAF* [16]. In addition, mutations of genes encoding key players of the phosphoinositide 3-kinase

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(PI3K) pathway, such as *PTEN*, *PIK3CA*, and *AKT1*, have been reported in PTC at lower frequencies [16]. Genetic alterations encountered in FTC include activating point mutations of *RAS*, present in about 45% of FTC; rearrangement of the paired-box gene 8 (*PAX-8*) with the peroxisome proliferator-activator receptor-γ (PAX8-PPARγ), observed in 35% of FTC; loss-of-function mutations of the tumor suppressor *PTEN* gene, encountered in about 10% of FTC; activating mutations or amplification of the *PI3KCA* gene, present in about 10% of FTC [17, 18].

Progression of DTC to PDTC and ATC implies tumor acquisition of novel genetic alterations, which are absent or present with low frequency in DTC tissues. Among these are mutations of the tumor suppressor gene *p53*, thought to be a gatekeeper of TC progression from the indolent DTC to the aggressive PDTC and lethal ATC [19]. In fact, *p53* mutations are rarely encountered in DTC (5–9% of cases), while increase in the PDTC (17–38% of cases) and ATC (67–88% of cases) [10, 20, 21]. A similar trend regards the *CTNNB1* gene, encoding the β-catenin, involved in cell adhesion and in the wingless (Wnt) signaling pathway [10]. In particular, *CTNNB1* gene mutations are not found in DTC, while they are present in PDTC (25% of cases) and ATC (66% of cases) [22, 23]. The conversion of early-stage TC to more aggressive and invasive malignancies occurs through an epithelial-to-mesenchymal transition (EMT), which implies the loss of cell-cell contacts, remodeling of cytoskeleton, and the acquisition of a migratory phenotype [24, 25]. Reduced expression of E-cadherin and abnormal expression of integrins, Notch, MET, TGFβ, NF-kB, PI3K, TWIST1, matrix metalloproteinases, components of the urokinase plasminogen-activating system and p21-activated kinase, all of them involved in the EMT, have been identified in TC progression [24–29].

Total thyroidectomy followed by adjuvant therapy with radioactive iodide ($^{131}$I) is the treatment of choice for the majority of patients affected by DTC [30]. Although the prognosis of these patients is favorable, with 10-year survival rate around 90%, about one-third of them face the morbidity of disease recurrence and TC-related deaths [30]. The worst outcomes are observed in patients with PDTC and ATC, in which the reduced expression of the thyroid-specific gene natrium/iodide symporter (NIS) renders $^{131}$I treatment useless [31–33]. In particular, patients affected by ATC have a dismal prognosis with a mean survival time of few months from the diagnosis [32]. Outcome of ATC patients is not influenced by current anticancer treatments (i.e., palliative surgery when possible, chemotherapy, and radiotherapy), and in the majority of cases, death occurs following tumor airway obstruction [34]. Thus, the identification of novel therapeutic approaches capable of improving PDTC and ATC patients' outcome is very much needed.

## 2. The Aurora kinases

The Aurora kinases belong to a family of serine/threonine kinases having in the Ipl1p (Increase in ploidy 1) gene, subsequently named Aurora gene, the founding member discovered in the budding yeast *Saccharomyces cerevisiae* during a genetic screening for mutations causing defective chromosomal segregation [35–38]. In mammals, the Aurora kinase family includes three proteins: Aurora-A, Aurora-B, and Aurora-C [39]. Structurally, they are characterized by

three domains: a N-terminal domain with little similarity among the three Aurora kinases, instrumental in determining their different intracellular localizations, substrate specificity and functions; a catalytic domain, containing the activation loop and highly related in sequence among the three proteins; and a short C-terminal domain of 15–20 residues (**Figure 1**). Aurora kinase expression is tightly regulated during cell cycle, being low in the G1/S phase and maximal in the G2/M phase.

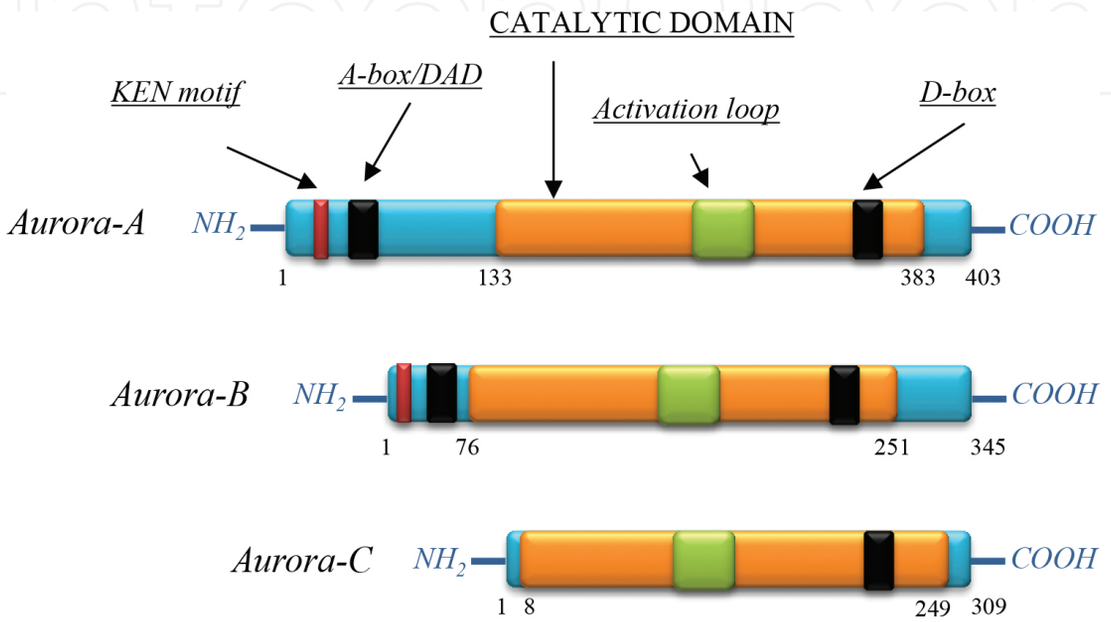


**Figure 1.** Schematic representation of Aurora kinase proteins. D-Box, destruction box; DAD, D box activating domain; KEN motif, amino acidic K-E-N, which serves as targeting signal for the Cdh1–anaphase promoting complex (adapted with permission from Ref. [14]).

### 2.1. Aurora-A

The Aurora-A is encoded by the *AURKA* gene located on the chromosome 20q13.2 and containing 11 exons (Gene ID: 6790). The *AURKA* promoter contains a putative TATA box at −37 to −14 and two CCAAT boxes at −101 to −88 and at −69 to −56 [Eukaryotic Promoter Database, Swiss Institute of Bioinformatics]. Two distinct cis-regulatory elements have been identified [40]. Of these, one positively regulates *AURKA* gene transcription, while the other is a cell cycle-dependent transcriptional repressor [40]. The former, essential for the gene expression, is a 7-bp sequence located at −85 to −79 that binds the transcription factor E4TF1. The second is formed by two repressor elements: a cell cycle-dependent element (CDE) located at −44 to −40, and a cell cycle gene homology region (CHR) located at −39 to −35 [40]. Over the last few years, a number of transcription factors capable of repressing or inducing *AURKA* gene expression have been identified. These include the p53, the HIF-1, and the INI1/hSSNF5, all reported to regulate negatively the activity of the *AURKA* promoter [41–43]. Conversely, other transcription factors have been shown to induce *AURKA* expression, among which the ΔEGFR/STAT5, the oncogene MYCN, and the MAPK via Ets2 transcription factors [44–47]. The

Aurora-A protein consists of 403 amino acids with a predicted molecular mass of 45.8 kDa (**Figure 1**). In the activation loop, an Aurora kinase signature (xRxTxCGTx) is present in which the autophosphorylation of the Thr288 is required for kinase activation [48]. In addition, the Thr288 is positioned within a protein kinase A (PKA) consensus sequence, and *in vitro* experiments indicated a potential role of PKA in Aurora-A phosphorylation [49, 50]. The phosphatase PP1 has been shown to dephosphorylate and inactivate Aurora-A [16]. The C-terminal located destruction box (D box), containing the motif RxxLxxG, and the N-terminal A-box/D-box-activating domain (DAD), containing the motif RxLxPS, play an essential role in Aurora-A degradation by the anaphase promoting complex/cyclosome (APC/C)-ubiquitin-proteasome pathway. Aurora-A degradation occurs in late mitosis/early G1 phase, when the D box is targeted by Fizzy-related proteins that transiently interact with the APC, and it is dependent from the APC/C activator protein Cdh1 [49–52]. In the N-terminal region the amino acidic sequence K-E-N, known as KEN motif, is also present, which serves as targeting signal for Cdh1-APC-mediated degradation of several mitotic proteins such as Nek2 and B99 [53]. However, this does not seem to be crucial for Aurora-A degradation [53]. Phosphorylation of the serine residue (Ser51) in the DAD domain has been shown to prevent Aurora-A degradation [54, 55].

### 2.2. Aurora-B

The Aurora-B is encoded by the *AURKB* gene mapped to chromosome 17p13.1, and consisting of nine exons (Gene ID: 9212). Its promoter contains three putative CAAT boxes at −99 to −86, at −66 to −53, and at −30 to −17 [Eukaryotic Promoter Database, Swiss Institute of Bioinformatics]. As above described for the *AURKA* promoter, also the *AURKB* promoter possesses the CDE and CHR elements, thought to be responsible for the regulation of gene expression throughout the cell cycle [54]. *AURKB* promoter activity is positively increased by transcription factors such the ETS2 *via* ETS-binding sites present in its sequence [46, 47]. The 1.4 kb transcript encodes a protein of 345 amino acids with a predicted molecular mass of 39 kDa (**Figure 1**) [39]. As Aurora-A, Aurora-B protein is characterized by a catalytic domain, a C-terminal D box, and an N-terminal A box/DAD [49–53]. However, different from Aurora-A, Aurora-B is not degraded by the same ubiquitin ligase, but following its binding to the human proteasome $\alpha$-subunit C8 in a proteasome-dependent manner [55].

### 2.3. Aurora-C

The Aurora-C is encoded by the *AURKC* gene localized at chromosome 19q13.43 and consisting of seven exons (Gene ID: 6795). The *AURKC* promoter is much less characterized with respect to those of Aurora-A and Aurora-B. A CCAAT box is present at −36 to −23 (Eukaryotic Promoter Database, Swiss Institute of Bioinformatics). *AURKC* promoter activity has been shown to be downregulated by the transcription factor PLZF [56]. The 1.3 kb transcript encodes a protein of 309 amino acids with a predicted molecular mass of 35.6 kDa (**Figure 1**) [39]. Different from Aurora-A and Aurora-B, Aurora-C does not contain the KEN and the A box/DAD motifs in its N-terminal region, while the C-terminal D box is present. The mechanism(s) underlying its degradation, however, still remains to be elucidated.

## 3. Expression, subcellular localization, and functions of the Aurora kinases

The Aurora kinases play a major role during mitosis [49, 50]. As mentioned above, these proteins display distinct intracellular localizations, substrate specificity and functions, and their expression and activity are tightly regulated at the transcriptional or posttranscriptional level, through phosphorylation/dephosphorylation and protein degradation [57].

### 3.1. Aurora-A

The expression of Aurora-A is cell cycle regulated, being very low during the G1-phase and starting to accumulate at the centrosome in the late S phase to be maximal at the G2-M transition. In this period, it localizes at the spindle poles, and it is degraded before cytokinesis [50, 53]. Aurora-A regulates centrosome separation and maturation, mitotic entry, and bipolar spindle formation. Recruitment of Aurora-A to the centrosome is controlled by the centrosome

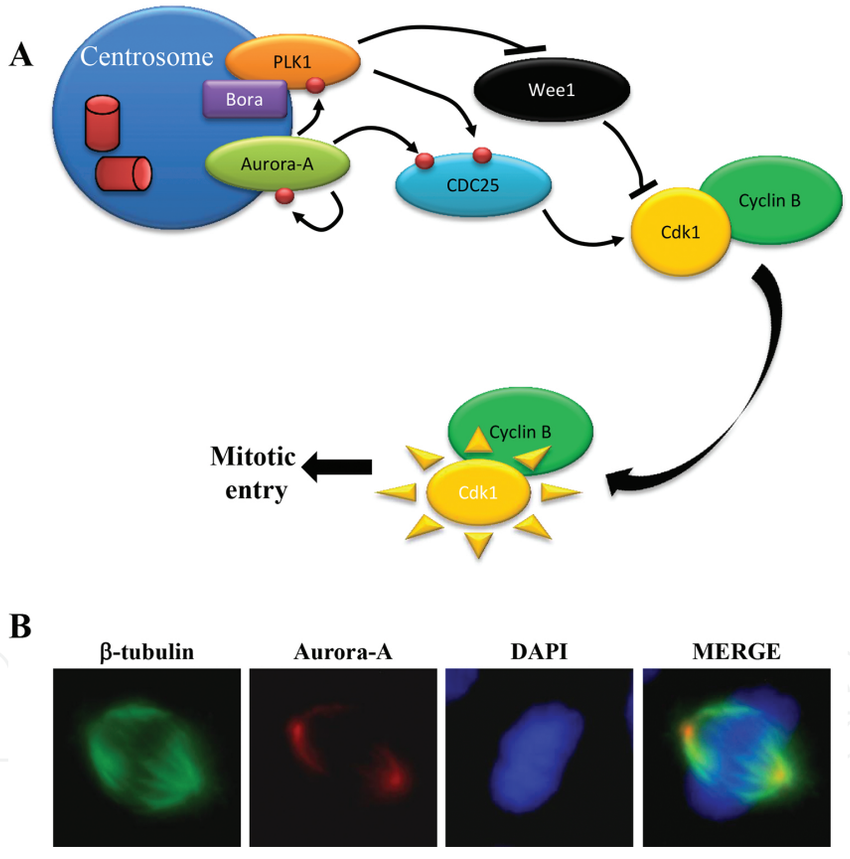


**Figure 2.** (A) Schematic representation of the pathway induced by Aurora-A to activate the CDK1/cyclin B complex allowing the transition of the cell from the G2 to the M phase. Aurora-A in association with Bora phosphorylates the PLK1. Both Aurora-A and PLK1 phosphorylate CDC25B (cell division cycle 25 B) allowing cyclin-dependent kinase 1 (CDK1)/cyclin B complex activation and thus promoting mitotic entry. PLK1 facilitates this process also by inhibiting the CDK1 inhibitor WEE1. Inactivation of Aurora-A or Plk1 individually shows no significant effect on Cdk1 activation and entry to mitosis, while their simultaneous inactivation produces a marked delay in both Cdk1 activation and mitotic entry, suggesting that the two kinases have redundant functions. (B) Immunofluorescence showing Aurora-A localization at the spindle pole of an anaplastic thyroid cancer cell in metaphase. Adapted with permission from Ref. [14].

protein of 192 kDa/spindle defective 2 (Cep192/Spd-2) [58]. On the centrosome, Aurora-A promotes the concentration in the pericentriolar mass of a number of proteins required for centrosome maturation and function. These include centrosomin, γ-tubulin, large tumor suppressor, homolog 2 (LATS2), transforming acidic coiled coil 3 (TACC3), and nuclear distribution element-like 1 (NDEL1) [50, 53, 59]. A central role of Aurora-A during mitosis is that to support the microtubule-organizer center (MTOC) responsible for the formation of the bipolar spindle. In this context, Aurora-A has been shown to form complexes with TACC1 and TACC3, which in turn, by binding to ch-TOG/XMAP215 proteins, stabilize microtubules at the centrosome [60–62]. In addition, Aurora-A interacts with and phosphorylates TPX2, which is capable of promoting spindle microtubule polymerization [53].

Aurora-A, along with the polo like kinase 1 (PLK1), controls the G2 to M phase transition (**Figure 2**) [63–65]. First, Aurora-A in association with the Bora protein phosphorylates the PLK1, after which both Aurora-A and PLK1 phosphorylate the cell division cycle 25 B (CDC25B), a member of the CDC25 family of phosphatases, which activates cyclin-dependent kinases by removing two phosphate groups, leading to CDK1/cyclin B complex activation and finally promoting mitotic entry [50, 63–66]. PLK1 facilitates this process also by inactivating the CDK1 inhibitor WEE1 (**Figure 2**).

### 3.2. Aurora-B

Aurora-B protein level peaks at G2/M phase, with the highest kinase activity recorded from metaphase to the end of mitosis [49, 50]. Aurora-B acts in concert with three other proteins, inner centromere protein (INCENP), Survivin, and Borealin/Dasra B, to which it associates forming the chromosomal passenger complex (CPC). In early prophase, the CPC is located on chromosomal condensing arms where it displaces the heterochromatin protein-1 from DNA

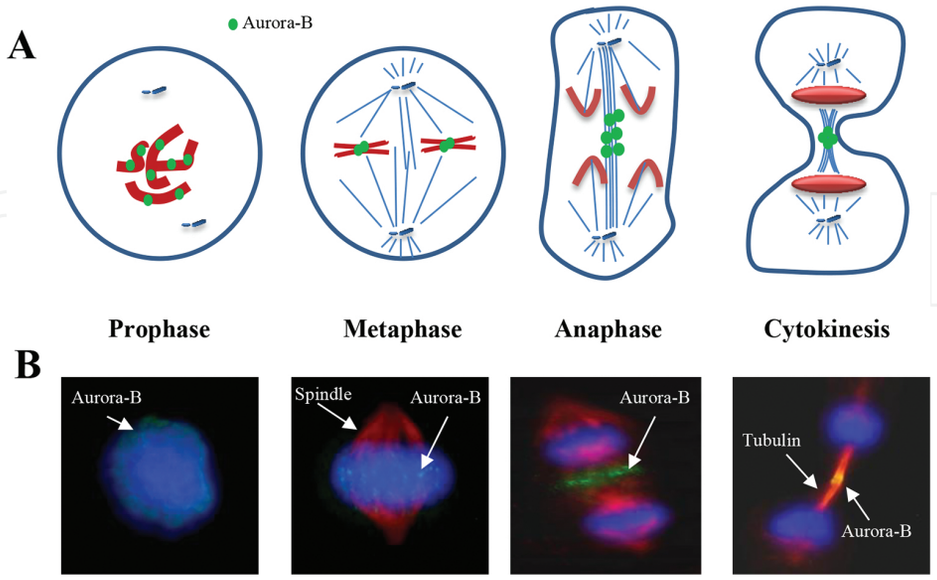


**Figure 3.** Schematic representation (A) and immunofluorescence images (B) of Aurora-B localization during mitosis in an anaplastic thyroid cancer cell. In (B) Aurora-B is in green, microtubules in red and DNA, stained by DAPI, in blue. Adapted with permission from Ref. [14].

to recruit condensin proteins (**Figure 3**) [67, 68]. From early G2 phase to prophase, Aurora-B phosphorylates histone H3, but its physiological meaning remains unclear. From late prophase to metaphase CPC localizes to the inner centromere, playing a role in formation and stability of the bipolar mitotic spindle, kinetochore assembly, correction of non-bipolar chromosome-spindle attachments, and control of the spindle checkpoint (**Figure 3**). In anaphase, the CPC relocates to the midzone of the mitotic spindle and to the cell cortex, remaining evident in the midbody of telophasic cells where it modulates the activity of several proteins involved in spindle dynamics, cleavage furrow formation and completion of cytokinesis (**Figure 3**) [49, 50, 67–69].

Aurora-B activation requires the auto-phosphorylation and binding to INCENP, while all CPC components are necessary for its proper localization during mitosis. Several kinases, such as BubR1 and Bub1 (checkpoint kinases), monopolar spindle 1 (Mps1), checkpoint kinase 1 (Chkl), Tousled-like kinase-1, Plk1, and TD-60/RCC2 (regulator of chromosome condensation 2), have been shown to be involved in Aurora-B activation. The phosphorylation status and activity of Aurora-B are controlled by PP1 and PP2A phosphatases [69].

### 3.3. Aurora-C

The expression of Aurora-C is maximal during the G2/M phase. This kinase seems to have a prominent role in the meiotic division, as it is expressed at relative high levels in germ cells during spermatogenesis and oogenesis, and at very low levels in somatic cells. Aurora-C is highly similar to Aurora-B in sequence (61% identity), which may explain why the two kinases display similar localization patterns and share interacting proteins and substrates such as INCENP, Survivin, Borealin, and others [49, 70]. Interestingly, when ectopically expressed in cells depleted of Aurora-B, Aurora-C is capable of rescuing the Aurora-B-dependent mitotic functions [40]. It is also worth to note that Aurora-C has been shown to interact with and phosphorylate TACC1 in thyroid cells in the cytokinetic bridge [71, 72].

## 4. Aurora kinases and cancer

Chromosomal instability is thought to represent the mean by which premalignant cells acquire novel functional capabilities responsible for cancer cell growth and tumor progression [73]. In fact, aberrations in chromosome number and structure, likely resulting from a combination of ineffective checkpoints and anomalous cellular divisions, occur in the majority of human cancers [74]. Given the crucial tasks of Aurora kinases in all mitotic stages, their dysfunction and/or dysregulation is believed to greatly contribute to aneuploidy. Whether Aurora kinases may have a role in cancer initiation is still a matter of debate. It has been reported that the overexpression of either Aurora-A, Aurora-B, or Aurora-C induces cell malignant transformation [75–77]. In different studies, however, the transforming ability of either Aurora-A or Aurora-B overexpression could not be confirmed [78, 79].

Aurora-A kinase has been often implicated in cancer progression, and its hyperactivation has been demonstrated to induce resistance to microtubule-targeted chemotherapy [80–82]. The

*AURKA* gene is amplified in many malignancies, and its overexpression has been reported to be significantly associated with a higher tumor grade and a poor prognosis in a number of cancers, including chondrosarcoma, nasopharyngeal carcinoma, breast cancer, glioblastoma, colorectal cancer, gastric cancer, and ovarian carcinoma [83–89]. In addition, somatic mutations located within the catalytic domain of Aurora-A, altering kinase activity and subcellular localization, have been described in human cancer cells [90]. The oncogenic potential of Aurora-A derives from a sum of several spatially and temporally distinct actions. Unlike normal cells, in many cancer cells the expression of Aurora-A becomes constitutive throughout the cytoplasm, regardless of the cell cycle phase; this can trigger a plethora of improper interactions, phosphorylations, and mislocalizations. Aurora-A may also represent the downstream target of mitogenic pathways, such as MAPK/ERK (mitogen-activated protein kinases), and be overexpressed because of their constitutive activation in tumors [81]. The Aurora-A excess interferes with different cell cycle checkpoints, that is, the late G2 checkpoint, which restrains genetically aberrant cells to enter mitosis, the spindle assembly checkpoint, which blocks the metaphase–anaphase transition in cells with defective spindles, and the post-mitotic G1 checkpoint, which arrests cell cycle in aneuploid cells [81, 83]. Centrosome amplification and unrestrained multinucleation, leading to abnormal mitotic spindle, are also observed in Aurora-A overexpressing cells [91]. Moreover, Aurora-A may significantly contribute to tumor progression by interacting with and inhibiting several tumor suppressor proteins such as p53, BRCA1 (breast cancer 1), and Chfr (checkpoint with forkhead and ring finger domains). Interestingly, activation of the MAPK signaling pathways has been found to induce accumulation of Aurora-A kinase in ER$\alpha^+$ breast cancer cells, and epithelial-to-mesenchymal transition (EMT) [92, 93]. In these cells, Aurora-A has been shown to promote SMAD5 phosphorylation and nuclear translocation, upregulation of stemness gene SOX-2, and acquisition of a stem cell-like phenotype [92, 93].

Aurora-B plays a less clear role in tumorigenesis. An increased level of Aurora-B in normal cells induces premature chromosome separation and segregation errors, promotes generation of tetraploid/aneuploid cells, and potentiates Ras oncogenic activity [77, 80, 94–96]. Neither amplification nor specific mutations of *AURKB* gene have been shown to occur in human malignancies; nevertheless, Aurora-B overexpression has been demonstrated in several cancer types, including hepatocellular and oral squamous cell carcinomas, where it correlates with poor prognosis [80, 94–96].

At present, very little is known about the role of Aurora-C in cancer progression. Although Aurora-C is almost not detectable in normal somatic cells, it is highly expressed in various tumor cell lines [97–100]. One study has described the transforming potential of overexpressed Aurora-C in NIH-3T3 cells, and a correlation between the level of active kinase and tumor aggressiveness of the cells injected in nude mice [77].

### 4.1. Aurora kinase inhibitors

The overexpression of Aurora kinases in human cancers and their relevance in controlling the mitotic process have led to the development of small-molecule inhibitors as anticancer drugs. Aurora inhibition results in cytokinesis failure and generation of tetraploid cells,

which, depending on the post-mitotic checkpoint activation, may be unable to proceed in a new cell cycle or rather may proliferate and become polyploid. The exit from cell cycle is likely to generate viable quiescent cells, whereas endoreplicating cells have greater tendency to undergo apoptosis. Actually, the functional inhibition of Aurora kinases is considered a promising therapeutic option against those malignancies that do not respond to the available therapies [101–108]. Up to date, about 30 small-molecule inhibitors of Aurora kinases have been developed and some of them, reported in **Table 1**, are being evaluated in Phase I-II clinical trials [101–108]. Of some interest are the preclinical observations showing the ability of different Aurora kinase inhibitors to have additive or synergist effects when combined with other anticancer therapies [109, 110]. At example, among the pan-Aurora kinase inhibitors, the AMG-900 in combination with the HDAC (histone deacetylase) inhibitor vorinostat has been shown to synergistically reduce proliferation and survival of medulloblastoma and prostate cancer-derived cell lines [111, 112]. Similarly, the SNS-314 has been shown to possess additive inhibitory effects on the HCT 116 cell line when combined with either carboplatin, gemcitabine, 5-FU, daunomycin, docetaxel, or vincristine [113]. Also the MK-0457 has revealed additive effects when combined with docetaxel on ovarian cancer cell lines or with cisplatin on the HepG2 cell line [114, 115]. Finally, the pan-Aurora kinase inhibitor CCT 137690 has been shown to sensitize SW620 colorectal cancer cells to radiotherapy [116]. In clinical trials, disease stabilization and, less frequently, partial responses in patients with solid cancers have been witnessed with the majority of Aurora kinase inhibitors, while more encouraging observations have been made in patients with hematological malignancies [101–110]. On-target toxicity observed with these drugs included grade 3/4 neutropenia, leukopenia, and myelosuppression, while off-target effects included hypertension, somnolence, mucositis, stomatitis, proctalgia, and ventricular dysfunction [101–110]. For example, the MK-0457 has been employed in different clinical trials in which patients with advanced solid tumors have been enrolled. In a Phase I dose escalation study, the most common dose-limiting toxicity observed was neutropenia and herpes zoster, and major adverse events include nausea, vomiting, diarrhea, and fatigue [117]. Although no objective tumor responses were observed in this trial, 12 of 27 patients experienced stable disease with a median duration of 75.5 days (range 38–328 days). Of the latter, one patient with ovarian cancer achieved prolonged stable disease for 11 months, and one patient with rectal cancer had stable disease over 7 months [117].

The MK-0457 was found to have off-target inhibitory effects on both wild-type and mutant Abl kinases and showed to be a potent inhibitor of the BCR-ABL T315I mutant, which mediates clinical resistance to imatinib, nilotinib, and dasatinib [118]. On these bases, a phase I/II dose escalation study of MK-0457 was performed in patients with leukemias [119, 120]. Patients with refractory hematologic malignancies received 1–21 cycles of MK-0457, and maximum-tolerated doses were calculated for a 5-day short infusion as 40 mg. Mucositis and alopecia were the most common drug-related adverse events observed in these patients. Forty-four percent (8/18) of patients, positive for the BCR-ABL T315I mutation, affected by chronic myelogenous leukemia (CML) had hematologic responses, and 33% (1/3) of patients with Philadelphia chromosome-positive (Ph+) acute lymphoblastic leukemia (ALL) obtained complete remission [119, 120].

| Inhibitor (company) commercial name | Clinical trial |
|---|---|
| **Pan-Aurora inhibitors** | |
| VX-680/MK-0457 (Vertex/Merck) Tozasertib | Phase II (terminated due to severe toxicity) |
| PHA-739358 (Pfizer/Nerviano) Danusertib | Phase II |
| PHA-680632(Pfizer/Nerviano) | Phase II |
| CYC-116 (Cyclacel) | Phase I |
| SNS-314 (Sunesis) | Phase I |
| R763 (Rigel) | Phase I |
| AMG-900 (Amgen) | Phase I |
| AT-9283 (Astex) | Phase II |
| PF-03814375 (Pfizer) | Phase I |
| GSK1070916 (GlaxoSmithKline) | Phase I |
| **Aurora-A inhibitors** | |
| MLN8237 (Millennium) | Phase II |
| EMD-2076 (EntreMed) | Phase II |
| MK-5108 (Vertex) | Phase I |
| **Aurora-B inhibitors** | |
| AZD1152 (AstraZeneca) | Phase II |

**Table 1.** Aurora kinase inhibitors in clinical trials (adapted with permission from Ref. [14]).

Another multicenter phase II study evaluated the safety and efficacy of MK-0457 on 52 patients affected by CML or Ph+ ALL with BCR-ABL T315I mutation [121] (Seymour et al. 2014). Patients were treated with a 5-day continuous infusion of MK-0457 administered every 14 days at 40, 32, or 24 mg. The most common adverse events were neutropenia and febrile neutropenia. Eight percent (4/52) of patients achieved major cytogenetic response and 6% (3/52) had a complete or a partial response. Thirteen percent (2/15) of patients with chronic phase CML achieved complete hematologic response. None of the patients with advanced CML or Ph+ ALL achieved major hematologic response [121].

A comprehensive description of clinical trials performed with the different Aurora kinase inhibitors has been recently reported [109, 110].

## 5. Aurora kinases and thyroid cancers

Normal human thyrocytes express all three Aurora kinases in a cell cycle-dependent manner [98]. The expression of Aurora-A and Aurora-B in these cells is mainly regulated at the transcriptional level, while that of Aurora-C appears to be modulated at the posttranscriptional level [98]. An increased expression of all the Aurora kinases has been shown in various cell

lines originating from different epithelial thyroid tumor histotypes, compared with normal thyrocytes, as well as in DTC and ATC tissues, compared with normal matched tissues [60, 98, 122]. In addition, a study aimed to evaluate the gene expression profile in ATC identified *AURKA* as one of the most frequently and most strongly overexpressed genes in these tumors [123]. In fact, gain of chromosome 20q, where *AURKA* is located (20q13.2), is frequently encountered in ATC [124]. Based on these findings, the potential therapeutic value of Aurora kinase inhibition on the proliferation and growth of PTC and ATC cells has been evaluated in preclinical studies [125–130]. In particular, different pan-Aurora kinase inhibitors, including the MK-0457 (VX-680), the SNS-314 mesylate, and the ZM447439 have been evaluated *in vitro* [126–129]. These molecules were found to inhibit proliferation of ATC cells in a time- and dose-dependent manner and to impair cancer cell colony formation in soft agar. Cell cultures exposed to pan-Aurora inhibitors revealed an accumulation of tetra- and polyploid cells because of endoreplication events followed by the activation of caspase-3 and accumulation of a sub-G0/G1 cell population, both indicative of apoptosis [126–129]. Treated cells showed mitotic alterations consistent with the inhibition of Aurora kinases, including major impairment of centrosome functions, with abnormal spindle formation characterized by the presence of short microtubules, inhibition of histone H3 phosphorylation, and inability to complete the cytokinesis. The effects of a selective inhibition of either Aurora-A or Aurora-B have been also explored [125, 129, 131]. The selective inhibition of Aurora-B expression, by means of RNA interference, or function, by means of small-molecule compounds (e.g., AZD1152), has been reported to significantly reduce growth and tumorigenicity of ATC-derived cells, both *in vivo* and *in vitro* [125]. In the same way, functional inhibition of Aurora-A by MLN8054 in a panel of ATC-derived cell lines has been shown to block cell proliferation and to induce cell cycle arrest and apoptosis [129]. In xenograft experiments, the drug was capable of reducing tumor volume by 86% [129]. Interestingly, the combined treatment with MLN8054 and bortezomib, targeting the ubiquitin-proteasome system, showed additive effects on ATC-derived cell proliferation and apoptosis, compared with monotherapy [131]. More recently, pazopanib, a multi-target inhibitor of tyrosine kinases including the VEGFR (vascular endothelial growth factor receptor), shown to have impressive therapeutic activity in patients affected by radioactive iodine-refractory DTC, was tested in a phase II clinical trial on ATC patients [132, 133]. Despite several of them treated with pazopanib had a transient disease regression, no response evaluation criteria in solid tumors (RECIST) response was obtained [131]. Moreover, in a preclinical study on a panel of ATC-derived cell lines, pazopanib was found to potentiate the cytotoxic effects of paclitaxel *in vitro* and in xenograft experiments [134]. These pazopanib effects were attributed to an unexpected off-target inhibition of Aurora-A in ATC-derived cell lines. In fact, the same results were obtained when combining paclitaxel and MLN8237, a selective Aurora-A inhibitor. In the same study, the authors also showed that the combined administration of pazopanib and paclitaxel attained a marked and durable regression of lung metastasis in a single ATC patient [134].

In conclusion, the preclinical and clinical data so far available indicate that Aurora kinase inhibitors may have a therapeutic potential for the treatment of the more aggressive thyroid cancers either in monotherapy or, more likely, in combination therapy with antimicrotubule drugs.

## Author details

Enke Baldini, Chiara Tuccilli, Salvatore Sorrenti, Domenico Mascagni, Stefano Arcieri, Angelo Filippini and Salvatore Ulisse*

*Address all correspondence to: salvatore.ulisse@uniroma1.it

Department of Surgical Sciences, "Sapienza" University of Rome, Rome, Italy

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