

Characterization of Aurora A and Its Impact on the Effect of Cisplatin-Based Chemotherapy in Patients with Non-Small Cell Lung Cancer



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Abstract

BACKGROUND AND OBJECTIVE: Aurora A, as a member of serine/threonine kinase family and a common characteristic of epithelial cancers, plays a critical role in cell mitosis. However, the clinical significance of Aurora A in non-small cell lung cancer (NSCLC) remains undetermined. **METHODS:** The expression of Aurora A in NSCLC and paired normal adjacent lung tissues was determined by immunohistochemistry, Western blot, and reverse transcriptase polymerase chain reaction. Receiver operating characteristic (ROC) curve analysis was employed to determine a cutoff score for Aurora A expression in a training set ($n = 135$). For validation, the ROC-derived cutoff score was subjected to analysis of the association of Aurora A expression with patient outcome and clinicopathological characteristics in a testing set ($n = 128$) and overall patients ($n = 263$). The correlation of Aurora A with cisplatin resistance and epithelial-mesenchymal transition (EMT) was examined *in vitro* in NSCLC cells by overexpression or knockdown of Aurora A. **RESULTS:** Aurora A expression was significantly upregulated in tumor tissues compared with paired normal tissues ($P < .01$). The expression of Aurora A was closely associated with clinical stage, lymph node metastasis, and recurrence and was an independent prognostic parameter in multivariate analysis. High level of Aurora A expression predicted poorer overall survival and disease-free survival in NSCLC patients treated with cisplatin-based adjuvant chemotherapy. *In vitro* data showed that overexpression or knockdown of Aurora A resulted in increased or decreased cellular resistance to cisplatin. Furthermore, inhibition of Aurora A reversed the EMT process. **CONCLUSIONS:** Aurora A was identified as an inferior prognostic and cisplatin-resistant biomarker in NSCLC patients, which provided potential evidences for therapeutic target and reversing drug resistance.

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Introduction

Lung cancer is the leading cause of cancer-related deaths [1,2]. Non-small cell lung cancer (NSCLC) accounts for 80% to 85% of lung cancers [3]. Most of the patients are diagnosed with advanced-stage disease, and the average 5-year survival rate remains dismal [4]. Multiple genetic abnormalities, including tumor suppressor genes, oncogenes, cell adhesion molecules, cell-cycle regulators, and growth factors, are responsible for the development and progression of NSCLC [5]. Molecular epidemiological studies have provided evidence that multiple genetic alterations make risk assessment of lung cancer patients more accurate. Aberrant proliferation of NSCLC is frequently associated with mutational activation of receptor tyrosine kinases signaling including genes encoding transmembrane receptor

tyrosine kinases (ALK [6], EGFR [6,7], ROS1 [8]) or intracellular signaling proteins such as KRAS [9] or its effectors A- B- or C-RAF [10] or PIK3CA [11]. BRAF is estimated to be mutated in ~2% of

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NSCLCs, 25% of which express the BRAF^{T1799A} oncogene encoding the BRAF^{V600E} oncoprotein kinase [12,13]. However, like many cancers, mutational activation of proto-oncogenes such as ERBB1, KRAS, or BRAF is generally accompanied by silencing of tumor suppressor genes such as TP53, CDKN2A, or PTEN that cooperatively serve to promote the stepwise malignant transformation of normal lung epithelial cells to malignant lung cancer cells [13]. Although much is known about the causal factors, clinical features, and pathogenesis of NSCLC, the molecular marker that has major clinical prognostic predictive value remains substantially limited. Thus, it is of great clinical value to further identify more valuable prognostic biomarkers.

Mitosis is a hallmark of epithelial cancers, raising the possibility that regulators of relative kinases have a role in tumorigenesis. The Aurora kinases play a key role in mitosis [14]; in particular, Aurora kinase A (Aurora A) is involved in various mitotic events, such as centrosome function and maturation, spindle assembly, chromosome alignment, and mitotic entry [14,15]. In cells, Aurora A expression and kinase activity are increased during late G2 to M phase, and its subcellular localization dynamically changes during the cell cycle [16]. Overexpression or amplification of Aurora A has been noted across a range of different tumor types, such as colon, breast, bladder, ovarian, and pancreatic cancers, and is linked with tumor progression and poor prognosis [17–19]. Furthermore, previous studies showed that overexpression of Aurora A increases migration and leads to resistance to chemotherapeutics [20,21]. Inhibition of Aurora A resulted in abnormal spindle formation, mitotic defects, and cell death, which serves as a promising target in cancer therapy, and several small-molecule inhibitors for Aurora A kinase are currently being investigated within clinical trials [22–24]. Thus, inhibition of Aurora kinase A is a rational target for anticancer treatment.

The aim of the present study was to investigate the expression of Aurora A in NSCLC specimens and determine its correlation with clinical characteristics. The current results showed that Aurora A, as detected by immunohistochemistry, was significantly higher in NSCLC tissues compared with the adjacent normal tissues and closely associated with tumor recurrence rate. High expression of Aurora A predicted an inferior overall survival (OS) and disease-free survival (DFS) in NSCLC patients treated with cisplatin-based adjuvant chemotherapy. Moreover, multivariate analysis revealed that Aurora A was an independent prognostic factor for NSCLC.

Methods

Patients

A total of 283 primary NSCLC patients were initially recruited in our study. All patients underwent initial surgical resection from March 2003 to January 2013. We further screened patients using a strict eligibility criteria protocol as follows: microscopically pathologically confirmed NSCLC, without any distant metastatic diseases, no prior chemotherapy or radiation therapy history, and having over 5-year follow-up period. Smoking history was based on records at patients' first clinic visit, and having smoked more than 100 cigarettes in a lifetime was used to define smokers. Performance status was evaluated using the Eastern Cooperative Oncology Group criteria. Ultimately, 20 patients with loss of follow-up were not included in the present study, leading to 263 NSCLC patients subjected to further clinical and survival analysis.

Of the 263 NSCLC patients (median age, 48.0 years; range, 20–72 years), those with positive lymph node metastasis were treated with four to six cycles of cisplatin + vinorelbine adjuvant chemotherapies after surgical resection, whereas patients with negative lymph node metastasis

did not receive adjuvant chemotherapies. No adjuvant radiotherapy was administered to any of the patients after surgery. The cohort of 263 NSCLC patients was then randomly divided into training set ($n = 135$) and testing set ($n = 128$) by computer (SPSS 17.0 software). The histological and stage types were determined according to the classification of NSCLC by the World Health Organization and International Union Against Cancer Tumor-Node-Metastasis staging system. This study was approved by the ethics committee and Institutional Review Board of Beijing Cancer Hospital.

Tissue Microarray Construction

The tissue microarrays (TMAs) were constructed according to a method described previously [25]. Briefly, two representative core tissue biopsies (0.6 mm in diameter) were punched from represented NSCLC tissues and one cylinder with the same diameter from adjacent normal lung tissues. Multiple sections (5 μ m thick) were cut from the TMA blocks and mounted on the microscope slides.

Immunohistochemical Analysis

In brief, after deparaffinization, rehydration, antigen retrieval, and blocking, the TMA slides were incubated overnight at 4°C with a polyclonal antibody against human Aurora A (1:500; Cell Signaling Technology, No. 12100) in a moist chamber. Then slides were incubated in corresponding secondary antibody at room temperature for 30 minutes. Specimens were visualized through staining with 3,3-diaminobenzidine. Finally, the sections were counterstained with hematoxylin, dehydrated, and mounted. Negative controls were obtained by replacing the Aurora A antibody with a normal murine IgG. Known immunostaining-positive slides were used as positive controls.

Staining Evaluation

The brown granules in both nucleus and cytoplasm of Aurora A were considered as positive staining. The staining intensity was graded as follows: negative (score 0), bordering (score 1), weak (score 2), moderate (score 3), and strong (score 4). In addition, the staining extent was also graded into five levels according to the percentage of cells with elevated Aurora A staining, including negative (score 0), 0% to 25% (score 1), 26% to 50% (score 2), 51% to 75% (score 3), and 76% to 100% (score 4). Aurora A staining was assessed by two pathologists who were unaware of any clinical details related to the patients. The assessment was congruent in 87% (229 identical scores in 263 cases) of the cases, suggesting a highly reproducible scoring system. Discrepant cases were reviewed for a second time. The value was selected until both pathologists agreed with the result.

Selection of a Cutoff Score for Aurora A Expression

The receiver operating characteristic (ROC) curve analysis was subjected to the selection of Aurora A cutoff score for analysis of OS and DFS in the training set, as described previously [26]. ROC curve analysis has been shown to be reproducible to evaluate immunohistochemistry protein expression and to select biologically or clinically a relevant cutoff score with maximum sensitivity and specificity. ROC curve analysis was facilitated by dichotomizing the features of patients' outcome into survival [death versus others (censored, alive, or death from other causes)] and progression (local failure or distant metastasis).

Follow-Up

All patients had follow-up records of over 5 years. After the completion of therapy, patients were observed at 3-month intervals

during the first 3 years and at 6-month intervals thereafter. OS was defined as the time from the tumor resection to the date of death or when censored at the latest date if patients were still alive; DFS was defined as the time from the tumor resection to the date of disease relapse/progression or the date of death or when censored at the latest date.

Cell Lines and Cell Culture

The human lung adenocarcinoma cell lines H1299, A549, SK-MES, A427, H2170, H358, and H23 were obtained from Peking University Cancer Hospital. Cell lines were cultured in respective culture supplemented with 10% fetal bovine serum at 37°C in humidified 5% CO₂ incubator.

Transient Transfection

A549 cells were transfected with an empty vector or FLAG-Aurora A. The negative control siRNA and siRNA against Aurora A (5'-AUGCC-CUGUCUUACUGUCA-3') were synthesized by GenePharma Company (Shanghai) for H23 cells to reduce the expression of Aurora A.

Western Blot and Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) Analysis

Cells or tissues were ground and lysed with the RIPA buffer on ice before being subjected to protein gel blot analysis. The protein concentration was detected by the Bradford method. Equal amounts of cell and tissue extract were subjected to electrophoresis in sodium dodecyl sulfate polyacrylamide gel and transferred to nitrocellulose membrane for antibody blotting. The membrane was then blocked and incubated with mouse anti-β-actin antibody, rabbit anti-Aurora A antibody (1:1000, Cell Signaling Technology, No. 12100), and EMT Antibody Sampler Kit (1:1000, Cell Signaling Technology, No. 9782T). RT-PCR by a real-time PCR system (Applied Biosystems, Foster City, CA) was described previously [27].

ATPlite Cell Proliferation Assay

The stable cells were pooled and seeded into 96-well plates with 2000 cells per well in triplicate, along with the vector control cells. After 24-hour incubation, cells were treated with DMSO control or cisplatin (2.5, 5, 10 μM) for 48 hours and then subjected to ATPlite cell proliferation assay (Perkin-Elmer).

Statistical Analysis

ROC analysis was used to get an optimal cutoff score of Aurora A expression for survival analysis in the training set ($n = 135$). For validation, the relationship between Aurora A expression and OS and DFS was evaluated in the testing set ($n = 128$) and the overall patient cohort ($n = 263$). Relationship between Aurora A expression and clinicopathological variables was analyzed by the χ^2 test or Fisher's exact test. Kaplan-Meier analysis was employed to evaluate the relationship between Aurora A expression and OS and DFS. Differences in survival probabilities between patient subsets were assessed by the log-rank tests. The multivariate Cox proportional-hazards model was utilized to estimate the hazard ratios and 95% confidence intervals (CIs) for patient outcome. All P values quoted were two sided, and $P < .05$ was considered statistically significant. Statistical analysis was performed using SPSS v. 17.0 (SPSS, Inc., Chicago, IL).

Results

Patient Characteristics

A total of 263 patients with NSCLC were enrolled in this study from March 2003 to January 2013 at Beijing Cancer Hospital, and

Table 1. Patient Characteristics

Characteristics	No. of Patients ($n = 263$)	Percentage (%)
Age (years)		
Median	48	
Range	20-72	
Gender		
Male	181	68.8%
Female	82	31.2%
Smoking history		
Never smoker	152	57.8%
Smoker	111	42.2%
Histology		
Adenocarcinoma	120	45.6%
Squamous cell carcinoma	122	46.4%
Adenosquamous carcinoma	21	8.0%
Stage		
I	94	35.7%
II	69	26.3%
III	100	38.0%

their clinicopathologic characteristics are listed in Table 1. The patients, 181 males (68.8%) and 82 females (31.2% %), ranged in age from 20 to 72 years (mean 48 years). Histological examination was performed on formalin-fixed tissues in all cases, and tumors were diagnosed and classified according to the American Joint Committee on Cancer classification. Briefly, in the training set, there are 92 males and 43 females, with 48 cases of stage I, 39 cases of stage II, and 48 cases of stage III. Meanwhile, there are 89 males and 39 females in the testing set, with 46 cases of stage I, 30 cases of stage II, and 52 cases of stage III.

Aurora A Expression in NSCLC and Adjacent Normal Tissues

Immunohistochemistry was employed to examine the protein expression of Aurora A in primary NSCLC specimens and adjacent normal tissues. Aurora A staining was located in both nucleus and cytoplasm, predominantly in nucleus. Moreover, Aurora A was overexpressed in the NSCLC (Figure 1, A and A'), whereas normal paired adjacent tissues showed nearly negative expression (Figure 1, B and B').

Consistent with immunohistochemistry results, Western blot analysis revealed a similar finding in NSCLC and normal lung tissues (Figure 2A). For the RT-PCR results, we also observed that Aurora A overexpressed in NSCLC tissues, whereas it expressed negatively in normal lung tissues (Figure 2B).

Cutoff Score for Aurora A "Positive" Expression

To further assess survival analysis and avoid the problems of multiple cutoff point selection, ROC curve analysis was employed to determine a cutoff score for Aurora A expression in the training set. As shown in Figure 3, A and B, the Aurora A cutoff score for OS and DFS in the training set ($n = 135$) was 3.8 ($P < .001$) and 3.5 ($P < .001$), respectively. Thus, a score of 4 (>4 vs ≤ 4) for Aurora A expression was selected as the uniform cutoff point to distinguish NSCLC patients as having high or low expression.

Aurora A Expression and Clinical Features

The clinical features including age, gender, smoking history, CEA level, initial clinical stage, histology, differentiation, tumor stage, lymph node metastasis, recurrence, and Aurora A expression were summarized in Table 2. The ROC-derived Aurora A cutoff score of 4 derived from the training set successfully segregated the testing set into two subgroups. In brief, patients with a cutoff score >4 are

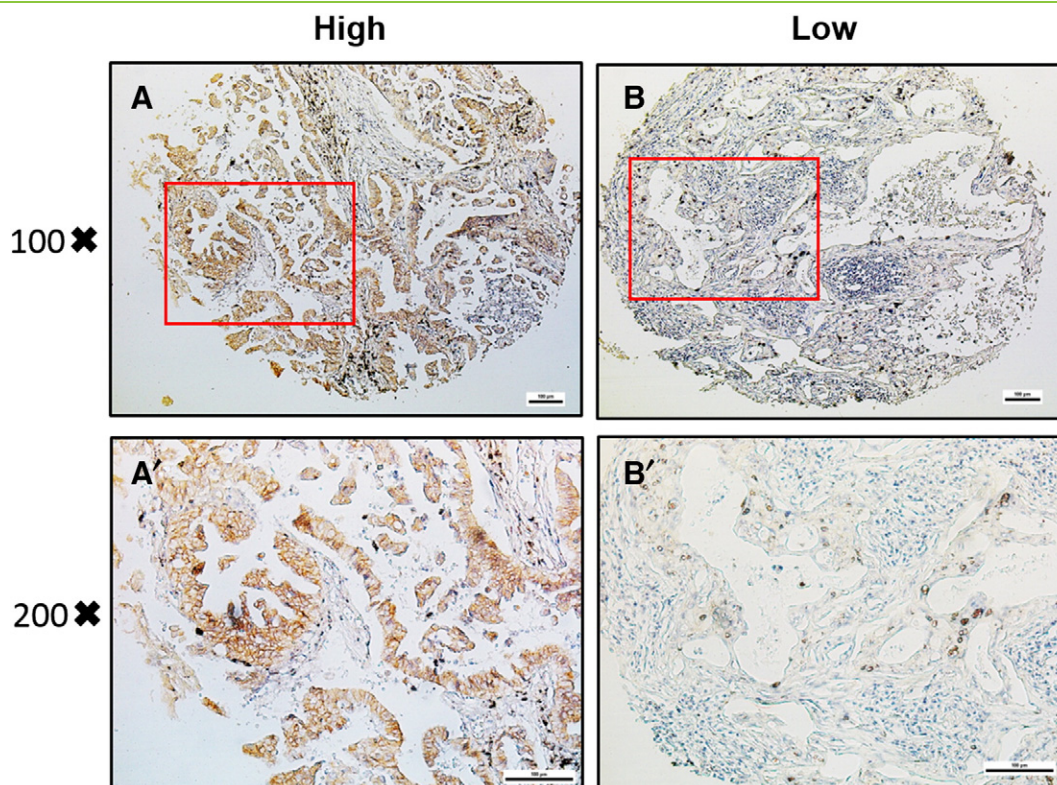


Figure 1. Aurora A expression in human NSCLC and adjacent normal tissue. (A) Aurora A was detected and overexpressed in the NSCLC tissue (100 ×). (B) Adjacent normal tissue showed nearly negative expression of Aurora A (100 ×). (A' and B') The higher magnification (200×) from the area of the box in A and B, respectively.

regarded as having Aurora A high expression (57/128, 44.5%), and those with a score ≤ 4 are viewed as having Aurora A low expression (71/128, 55.5%). High expression of Aurora A was mainly found in high CEA concentration (95/145, 65.5% in >5.0 ng/ml vs 33/118, 28.0% in ≤ 5.0 ng/ml, $P = .004$). High expression of Aurora A was mainly detected in patients with late clinical stage (71/100, 71% in patients with stage III vs 27/94, 28.7% in patients with stage I, $P < .001$). In addition, Aurora A expression also positively correlated with the initial tumor stage ($P < .001$). High expression of Aurora A was associated with metastatic spread to the lymph nodes ($P < .001$). Aurora A level also correlated with differentiation ($P = .012$) in the testing set. Furthermore, correlation analysis demonstrated that Aurora A expression was positively correlated with tumor recurrence in both sets ($P < .001$ in the training set and $P < .001$ in the testing set). We could not show any correlation between Aurora A expression and other patient characteristics including age, gender, smoking history, etc.

Aurora A Expression and Survival Analysis: Univariate Survival Analysis

As shown in Figure 4, Kaplan-Meier analysis showed that high expression of Aurora A strongly predicted an inferior OS and DFS in overall patients ($P < .001$ for OS and $P = .002$ for DFS, Figure 4, A and B). In the overall patient cohort, the median duration of overall survival for patients with high and low expression of Aurora A was 24.6 versus 78.8 months, respectively. Also, elevated expression of Aurora A predicted an inferior DFS with the median duration of DFS of 13.4 versus 56.8 months, respectively. Further analysis was performed between Aurora A expression and subsets of NSCLC patients within each clinical

stage. High expression of Aurora A also served as a poor prognostic factor in each stage of NSCLC patients in the testing set: stage I (median OS: 102 vs 48.5 months, median DFS: 68.4 vs 42.8 months, $P = .033$ for OS and $P = .024$ for DFS; Figure 5, A and B), stage II (median OS: 82.6 vs 40.0 months, median DFS: 58.0 months vs 22.5 months, $P = .026$ for OS and $P = .050$ for DFS; Figure 5, C and D), and stage III (median OS: 36.3 vs 11.2 months, median DFS: 9.8 vs 5.2 months, $P = .012$ for OS and $P = .047$ for DFS; Figure 5, E and F).

Multivariate Cox Regression Analysis

To avoid the influence caused by univariate analysis, the expression of Aurora A as well as other parameters was examined in multivariate Cox analysis (Tables 3 and 4). In the testing set, Aurora A was found to be a significant independent prognostic factor for poor OS (hazard ratio, 4.221; 95% CI, 2.493-6.920; $P < .001$; Table 3) and DFS (hazard ratio, 3.892; 95% CI, 1.027-5.237; $P < .001$; Table 3). Similar results were also observed in overall patients (hazard ratio, 4.271; 95% CI, 2.541-5.684; $P < .001$ for OS and hazard ratio, 3.789; 95% CI, 2.007-4.487; $P < .001$ for DFS; Table 4). Of other parameters, CEA and smoking history were found to be an independent prognostic factor for patient survival in the testing set and in overall patients.

Overexpression of Aurora A Increases Resistance and Epithelial-Mesenchymal Transition (EMT)

We sought to explore the association between Aurora A expression and cisplatin resistance *in vitro*. Aurora A expression was different in multiple human lung cancer cell lines, with low expression in A549 cell line and high expression in H23 cells (Figure 6A). Thus, FLAG-Aurora A markedly

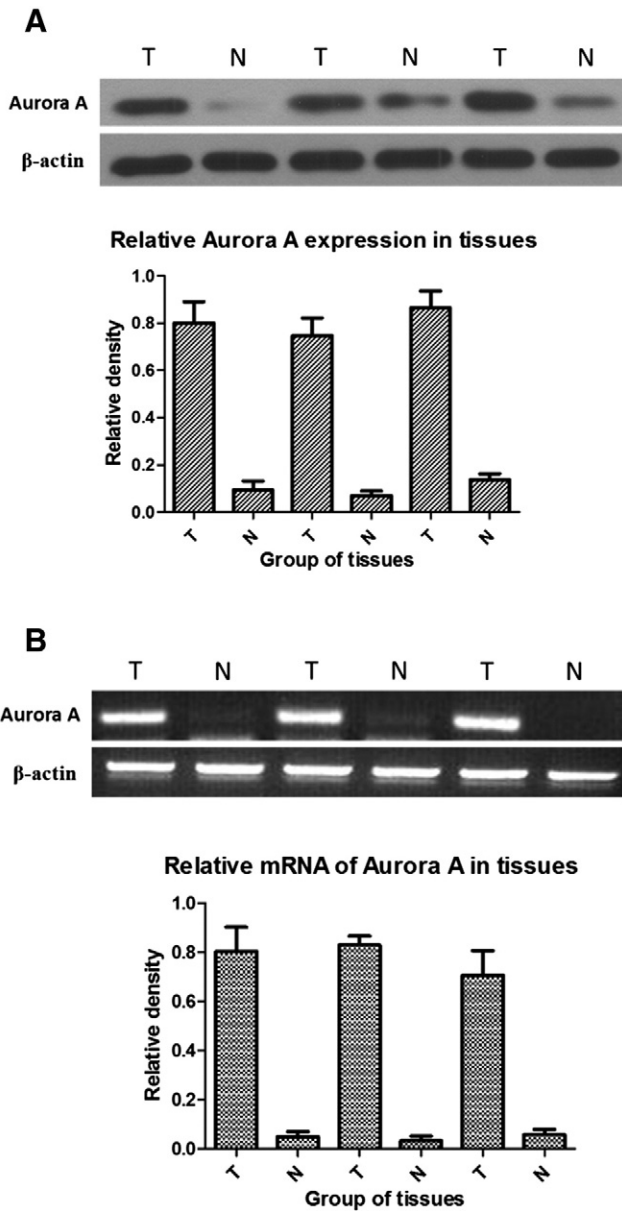


Figure 2. Aurora A expression in human NSCLC and adjacent normal tissue. (A) Western blot analysis of Aurora A expression in representative primary NSCLC tissue (T) and adjacent normal tissue (N). (B) The mRNA results were consistent with protein results.

increased the Aurora A expression in A549 cells, and siRNA knockdown of Aurora A caused the decreased expression in H23 (Figure 6B). The ectopic expression of Aurora A induced A549 cells resistant to cisplatin. However, knockdown of Aurora A could significantly sensitize H23 cells to cisplatin-induced growth suppression (Figure 6B). On the other hand, Aurora A was found to mediate EMT by detecting some related proteins, as evidenced by decreased levels of E-cadherin (E-cad) and increased levels of Snail and Slug (Figure 6C). Taken together, these results indicated that Aurora A induced cisplatin resistance and EMT process.

Discussion

Although treatment for NSCLC has improved in recent years with the development of targeted drugs for patients with amenable mutations, only a small proportion of patients have these mutations, and most tumors

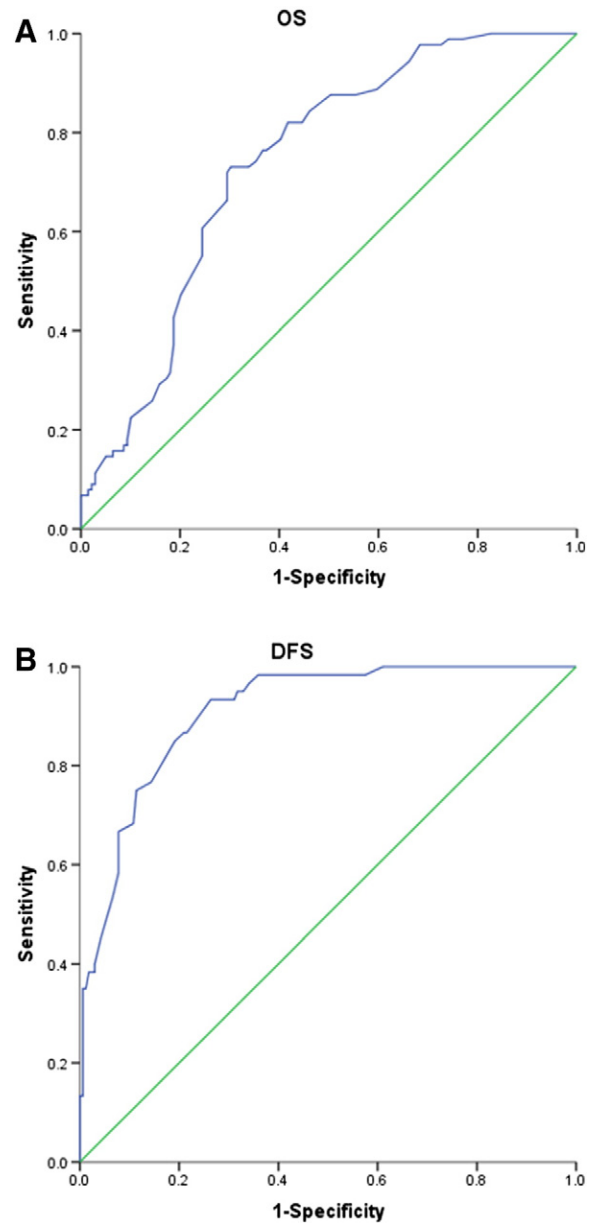


Figure 3. ROC curves analysis of Aurora A cutoff score in the training set. (A) Aurora A cutoff point for OS in the training set. (B) Aurora A cutoff point for DFS in the training set. At each immunohistochemical score, the sensitivity and specificity for the outcome being studied were plotted, thus generating an ROC curve. Aurora A cutoff score for OS and DFS was 3.8 and 3.5, respectively.

become resistant to targeted treatment [28–30]. Thus, more novel molecular markers that can identify tumor progression and predict the prognosis individually are still urgently needed. In the present study, we evaluated Aurora A expression in NSCLC, showing that, at both transcript and protein levels, Aurora A expression was significantly upregulated in NSCLC tumor samples compared with matched lung normal tissue. Furthermore, Aurora A overexpression was associated with poor survival in NSCLC patients. Aurora A overexpression decreased the sensitivity of lung cancer cells to cisplatin and induced EMT *in vitro*.

Genetic amplification and mRNA and protein overexpression of Aurora kinase A are implicated in the genesis of various neoplasms, which are significantly associated with aneuploidy, high tumor grade,

Table 2. Association of Aurora A Expression with Clinicopathologic Characteristics in NSCLC Patients

Variable	All Cases	Training Set (n = 135)			Testing Set (n = 128)		
		High	Low	P	High	Low	P
Age (years)							
≥62.0	129	34	30		29	36	
<62.0	134	37	34	.724	28	35	.689
Gender							
Male	181	50	42		38	51	
Female	82	21	22	.583	19	20	.325
Smoking history							
Yes	152	45	35		30	42	
No	111	26	29	.528	27	29	.315
CEA (ng/ml)							
>5.0	145	50	22		45	28	
≤5.0	118	21	42	.044	12	43	.025
Initial clinical stage							
I	94	16	32		11	35	
II	69	17	22		13	17	
III	100	38	10	<.001	33	19	.003
Histology							
Squamous cell carcinoma	120	37	34		21	28	
Adenocarcinoma	122	34	30	.452	27	31	.821
Adenosquamous cell carcinomas	21	0	0		9	12	
Differentiation							
Highly	62	17	12		6	27	
Moderately	94	28	20		18	28	
Poorly	107	26	32	.165	33	16	.012
Tumor stage							
T ₁ + T ₂	128	20	37		14	57	
T ₃ + T ₄	135	51	27	.031	43	14	.042
Lymph node metastasis							
Negative	137	26	48		17	46	
Positive	126	45	16	.011	40	25	.012
Recurrence							
Positive	132	51	15		40	26	
Negative	131	20	49	<.001	17	45	<.001

increased invasiveness, and poor prognosis [18,31,32]. Activation of the Aurora A kinase regulates cellular biological processes like centrosome maturation, entry into mitosis, formation and function of the bipolar spindle, and cytokinesis [16]. There are overwhelming evidences which report overexpression and gene amplification of Aurora A in several human cancers and suggest that Aurora A could be a bona fide oncogene involved in tumorigenesis. For instance, aberrant Aurora A mRNA and protein are common in various malignant tumors including breast [33], colon [34], ovarian [31], and pancreatic [18] cancers, indicating that it is important for tumor formation or progression. Also, Aurora A increases migration and leads to resistance to chemotherapeutic drugs [20,35]. Furthermore, numerous substrates of Aurora A kinase have been identified, which are predominantly related to cell cycle progression, whereas some of them are transcription factors. Aurora A-mediated phosphorylation can either directly or indirectly regulate the function of its substrates [36–38]. These properties have led Aurora A to be considered a high-value target for development of cancer therapeutics, with multiple agents currently in early-phase clinical trials [22,39]. Alisertib is an investigational, oral, selective inhibitor of Aurora kinase A. In xenograft models, this agent showed potent inhibition of Aurora kinase A and high antitumor activity across a range of tumor types [40]. Also, it is currently undergoing evaluation in a phase I/II trial in various nonhematological and hematological malignant diseases [23,41–43].

In this study, to develop an objective Aurora A cutoff score for survival analysis, we used the ROC curve analysis to generate a cutoff

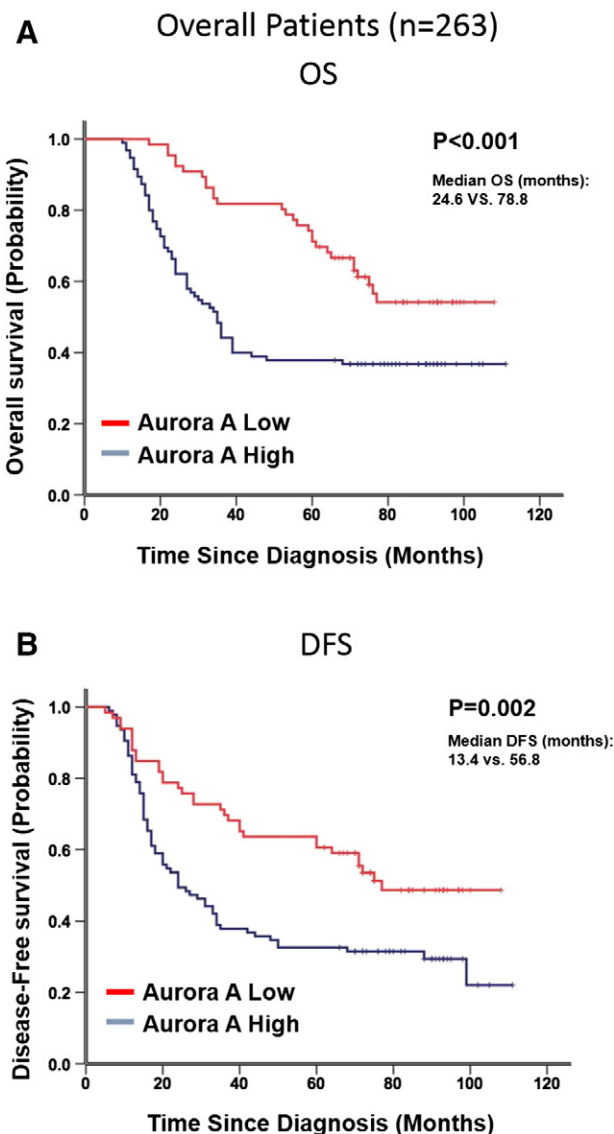


Figure 4. Kaplan-Meier survival analysis of Aurora A expression in overall patients. Patients with higher Aurora A expression acquired an inferior (A) OS and (B) DFS in overall patients. The median duration of OS and DFS for patients with high and low expression of Aurora A was 24.6 versus 78.8 months ($P < .001$) and 13.4 versus 56.8 months ($P = .02$), respectively.

score in the training set. Aurora A expression, which was classified as high and low level by the ROC-derived cutoff score, was mainly found to be higher in more advanced tumor stages, indicating that Aurora A might be involved in NSCLC progression. Correlation analysis further demonstrated that high Aurora A expression was associated with clinical stage and tumor invasion in NSCLC. Furthermore, in the testing set and overall patients, high Aurora A expression predicted a significant OS and DFS disadvantage over the low-Aurora A expression subgroup. Importantly, worse prognostic impact of increased Aurora A expression was demonstrated in patients with stage III and IV tumors, indicating that Aurora A might be a novel factor for risk definition in NSCLC. In addition, multivariate analyses in the testing set and overall patients revealed that Aurora A expression was an independent prognostic parameter. Taken together, our findings in this study provided evidence that overexpression of

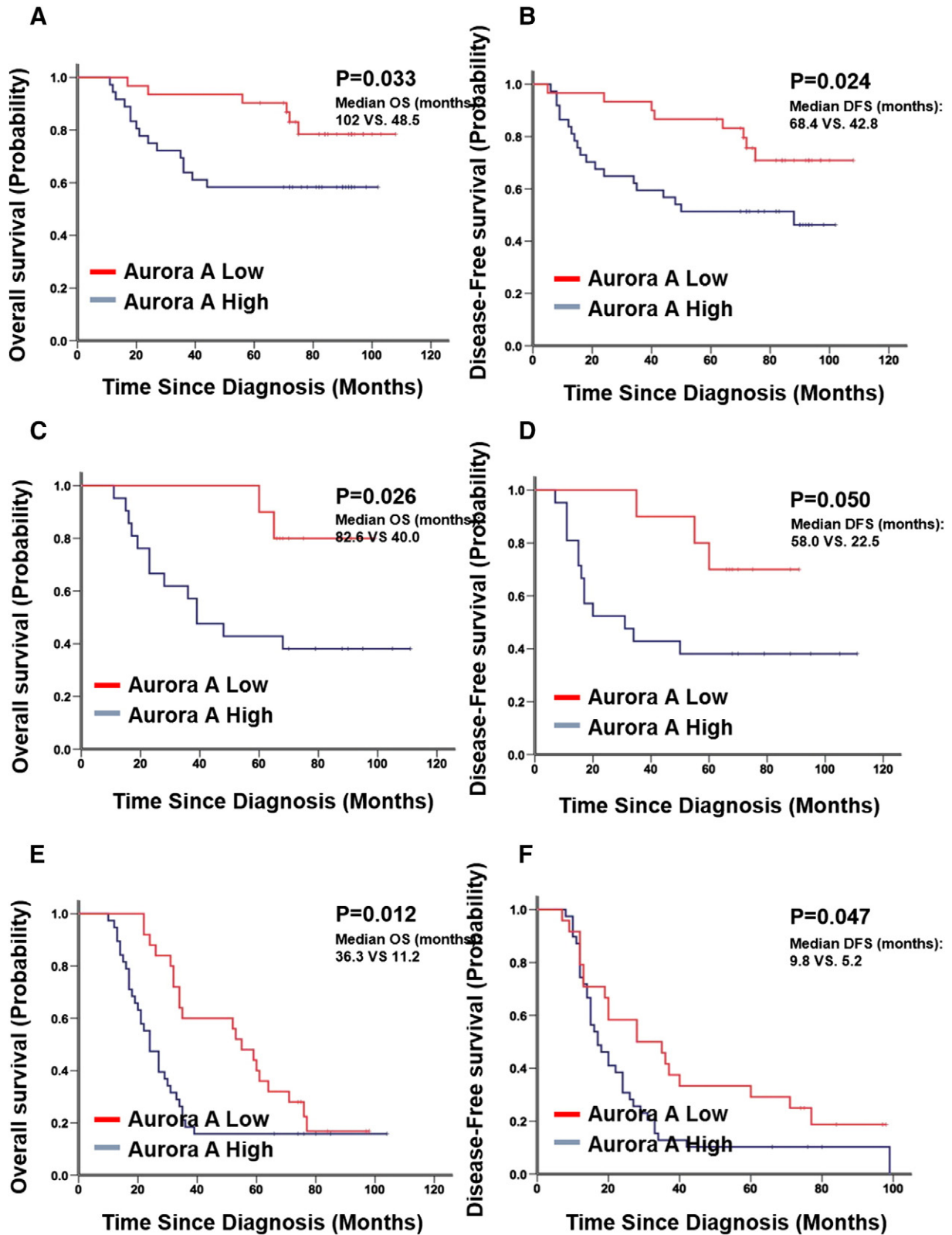


Figure 5. Kaplan-Meier survival analysis of Aurora A expression in subsets of NSCLC patients with stage I, II, and III disease (log-rank test). Probability of (A) OS and (B) DFS of patients with stage I NSCLC in the overall patients: low expression, $n = 67$; high expression, $n = 27$. Probability of (C) OS and (D) DFS of stage II patients with NSCLC in the overall patients: low expression, $n = 39$; high expression, $n = 30$. Probability of (E) OS and (F) DFS of patients with stage III NSCLC in the overall patients: low expression, $n = 29$; high expression, $n = 71$.

Aurora A in NSCLC might facilitate an increased malignant and worse prognostic phenotype of this tumor, which was consistent with the previous studies.

As for the underlying mechanism of Aurora A involved in chemoresistance, recurrence, and metastasis, it remains complicated and varies in various types of human cancers. Inhibition of Aurora A

Table 3. Results of Multivariate Cox Proportional-Hazards Analysis in Testing Set ($n = 128$).

Variable	For OS			For DFS		
	Hazard Ratio	95% CI	<i>P</i>	Hazard Ratio	95% CI	<i>P</i>
Age (years) ≥ 60.00 (vs <60.0)	1.134	(0.892-2.142)	.472	1.241	(0.784-2.024)	.812
Gender male (vs female)	2.532	(1.432-5.241)	.221	2.532	(1.893-4.342)	.032
Smoking history yes (vs no)	3.982	(1.993-5.326)	.003	2.143	(1.423-3.253)	.012
CEA (ng/ml) >5 (vs ≤ 5)	2.755	(1.435-3.253)	.001	2.532	(1.245-3.914)	.003
Initial clinical stage						
I	1.053	(0.298-3.143)	.462	1.336	(0.624-3.573)	.927
II	0.932	(0.422-2.986)	.765		(0.356-2.650)	.772
III	1	1		1	1	
Histology						
Squamous cell carcinoma	1.381	(0.386-5.334)	.622	1.142	(0.705-3.098)	.723
Adenocarcinoma	1.588	(0.672-5.924)	.325	1.724	(0.223-3.981)	.471
Adenosquamous carcinoma	1	1		1	1	
Differentiation						
Well	0.837	(0.472-1.987)	.342	0.434	(0.341-1.243)	.083
Moderately	1.232	(0.583-2.538)	.602	0.736	(0.315-1.430)	.277
Poorly	1	1		1	1	
Tumor stage T4 + T3 (vs T2 + T1)	1.098	(1.002-3.940)	.561	1.303	(1.002-2.874)	.026
Lymph node metastasis positive (vs negative)	2.906	(2.093-6.232)	<.001	3.092	(1.409-6.243)	.033
Aurora A low (vs high)	4.221	(2.493-6.920)	<.001	3.892	(1.027-5.237)	.001

in cells expressing mutant JAK2 abolishes the resistance to cisplatin [44] and enhances cisplatin-induced cell death in esophageal carcinoma cells [39,45]. It has been reported that Aurora A induces cisplatin chemoresistance by inhibition of p53, leading to downregulation of PTEN and activation of Akt in human ovarian cancer cells [46]. Some studies show that Aurora A promotes chemotherapeutic drug resistance via a NF- κ B signaling pathway in p53 knockdown lung cancer cells [47]. EMT is a malignant cancer phenotype characterized by aggressive invasion and metastasis, and resistance. Metastatic tumors are invariably more resistant to the chemotherapy compared with primary tumors as evidenced by the marked decrease of chemotherapy response rate [48,49]. Furthermore, recent studies have shown a relation between cisplatin resistance and the EMT phenotype [50]. Resistance to cisplatin was observed in cell lines undergoing EMT [50]. In our study, we found that Aurora A-related cisplatin resistance may be conferred by increasing of EMT. Thus,

therapeutic combinations using EMT signaling inhibitors may reverse the resistance of some types of cancer to chemotherapy.

In summary, our results provide a basis for the concept that increased expression of Aurora A in human NSCLC may be important in the tumor progression and serves as an independent biomarker for poor survival. Thus, overexpression of Aurora A identifies patients at high risk and is a novel therapeutic molecular target for NSCLC. Also, our data suggest that increased expression of Aurora A is related to cisplatin resistance. Consequently, inhibition of Aurora A kinase may be a promising regimen to overcome recurrence, metastasis, and resistance in NSCLC patients.

Acknowledgements

No conflicts of interests are present.

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Table 4. Results of Multivariate Cox Proportional-Hazards Analysis in Overall Patients

Variable	For OS			For DFS		
	Hazard Ratio	95% CI	<i>P</i>	Hazard Ratio	95% CI	<i>P</i>
Age (years) ≥ 60.00 (vs <60.0)	1.376	(0.953-1.999)	.092	1.041	(0.709-1.544)	.842
Gender male (vs female)	1.453	(0.832-2.563)	.257	2.532	(0.745-2.239)	.379
Smoking history yes (vs no)	1.496	(1.023-2.384)	.041	1.107	(0.648-1.927)	.421
CEA (ng/ml) >5 (vs ≤ 5)	2.188	(1.489-3.210)	<.001	2.046	(1.386-2.994)	<.001
Initial clinical stage						
I	0.768	(0.466-1.686)	.583	0.962	(0.345-2.625)	.915
II	0.846	(0.468-1.585)	.609	0.982	(0.474-1.427)	.761
III	1	1		1	1	
Histology						
Squamous cell carcinoma	1.293	(0.389-4.072)	.726	0.978	(0.345-2.782)	.723
Adenocarcinoma	1.072	(0.308-3.867)	.926	0.632	(0.198-1.098)	.742
Adenosquamous carcinoma	1	1		1	1	
Differentiation						
Well	0.756	(0.343-1.734)	.353	0.642	(0.389-1.129)	.132
Moderately	0.595	(0.319-1.584)	.085	0.913	(0.604-1.476)	.547
Poorly	1	1		1	1	
Tumor stage T4 + T3 (vs T2 + T1)	1.221	(0.704-1.762)	.613	1.159	(0.687-1.984)	.482
Lymph node metastasis positive (vs negative)	2.765	(1.726-3.856)	<.001	1.682	(1.268-2.748)	.007
Aurora A low (vs high)	4.271	(2.541-5.684)	<.001	3.789	(2.007-4.487)	<.001

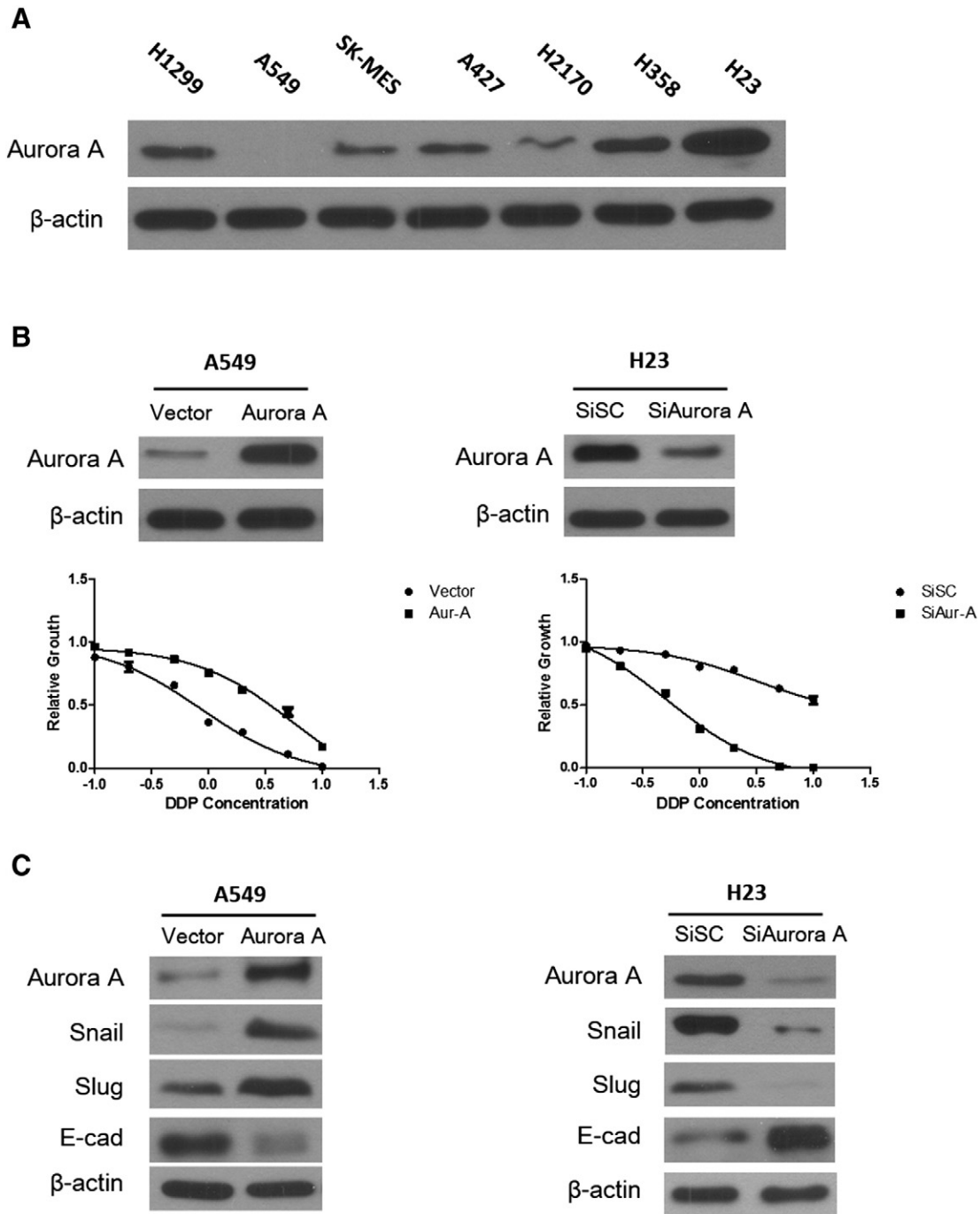


Figure 6. (A) Protein levels of Aurora A in multiple lung cancer cell lines. Cell lysates were prepared from indicated cell lines, followed by Western blotting with equal amount protein loaded, using indicated antibodies. (B) Overexpression of Aurora A induced cisplatin resistance in lung cancers, whereas Aurora A knockdown sensitizes lung cancer cells to cisplatin-induced apoptosis. (C) Overexpression of Aurora A contributed to increased Snail and Slug with decreasing E-cad, which indicated EMT process. However, knockdown of Aurora A upregulated E-cad but downregulated Snail and Slug, which inhibited EMT process.

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