

Original Article

Inhibition of Aurora A promotes chemosensitivity via inducing cell cycle arrest and apoptosis in cervical cancer cells

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Abstract: Aurora kinase A (AurA) regulates genomic instability and tumorigenesis in multiple cancer types. Although some studies have reported that Aur A may predict cervical cancer outcomes, its precise function and molecular mechanism in cervical cancer pathogenesis remain unclear. In this study, by overexpression or silencing of Aur A in cervical cancer cell lines, we found that overexpression of Aur A promoted cell proliferation through G1/S cell cycle transition and anti-apoptosis, xenograft tumor growth and chemoresistance to Taxol. We further found that inhibition of Aur A with its specific inhibitor VX-680 enhanced the antitumor effect of Taxol via inducing apoptosis. Moreover, the clinical analysis from tissue samples demonstrated that Aur A was overexpressed, and the expression of Aur A and pERK1/2 was negatively correlated in cervical cancer tissues. The above results may provide some potential insights in treatment of cervical cancer in clinic.

Keywords: Aurora A, VX-680, Taxol, chemoresistance, cervical cancer

Introduction

Cervical cancer is one of the most common neoplastic diseases affecting women worldwide, and remains a high cause of mortality among women in developing countries [1, 2]. After years' exploration, chemotherapy has been developed as one of the most useful strategies in systematic treatment of cervical cancer. Drugs used to treat cervical cancer are mainly through targeting some major cellular events, such as mitosis [3].

Mitosis, a highly dynamic phase of the cell cycle, is under precise control in lieu of several stringent determinants, such as spindle checkpoint to connect centromere to microtubule and chromosome alignment to ensure that sister chromosomes can be separated into daughter cells accurately [4]. The faults of mitotic checkpoint result in chromosomal abnormali-

ties and aneuploid cell subpopulations in various cancers [5]. One of the most important proteins involved in mitosis is Aur A, which is associated with centrosome maturation and spindle assembly. Aur A localizes to centrosomes during interphase and moves to the spindle poles during early mitosis [6]. Additionally, compelling evidence showed that Aur A was amplified or overexpressed in various tumors, making Aur A as a typical oncogene [7].

In view of cancer treatment, Aurora kinases have been highlighted as drug targets [8]. To date, a growing number of inhibitors targeting Aurora kinases have been described including Hesperidin, ZM447439, and VX-680 [9]. Literatures have revealed that VX-680 can block cell-cycle progression and induce apoptosis in many human tumor types, such as leukemia [10], prostate cancer [11], oral squamous cancer [12], ovarian cancer [13], suggesting

that VX-680 can be utilized as a potent therapeutic agent for carcinoma [14]. However, there is a paucity of evidence about VX-680 in its effect on cervical cancer cells. So we evaluated the effect of VX-680 on proliferation and apoptosis of cervical cancer cells in terms of overexpression or silencing of Aur A.

With the basic and clinical research advances rapidly, Taxol-based neoadjuvant chemotherapy (NACT) has greatly improved the prognosis of patients. However, due to the toxicity to normal tissues and the acquired resistance, the effectiveness and application of Taxol-chemotherapy is severely restricted. Therefore, looking for some agents that may synergistically increase the therapeutic efficacy of Taxol seems extremely necessary to improve the survival of cervical cancer patients. Since Aur A overexpression confers cell resistance to Taxol treatment [15], the use of Aur A inhibitors may reverse the resistance to Taxol.

To test our hypothesis, we stably transfected Aur A cDNA or shRNA into cervical cancer cells and investigated the function of Aur A and the chemotherapeutic efficiency of the Aur A inhibitor VX-680 in resulting cell lines. The results suggest that Aur A acts as an oncogene to promote cancer growth and that the Aur A inhibitor VX-680 can enhance the curative efficacy of Taxol in cervical cancer cells, which may provide a new idea to the treatment of cervical cancer.

Materials and methods

Cell lines and cell culture

Human cervical cancer cell lines SiHa, ME 180, and retroviral packaging cells (Phoenix amphotropic cells) were purchased from American Type Culture Collection (Manassas, VA). SiHa and Phoenix cells were maintained in DMEM medium, supplemented with 10% fetal bovine serum, 2 mM L-glutamine, nonessential amino acids (1%), 1 mM sodium pyruvate, penicillin (100 units/mL), and streptomycin (100 µg/mL). ME 180 cells were maintained in McCoy's 5A medium containing 10% fetal bovine serum, 2 mM L-glutamine, penicillin (100 units/mL), and streptomycin (100 µg/mL). All cells were cultured in monolayer at 37°C incubator with 100% humidity and 5% CO₂.

Chemicals

VX-680 and Taxol were purchased from Beyotime Biotechnology Corporation, Shanghai (Shanghai, China) and dissolved in DMSO at a concentration of 25 µM. The aliquots were stored at -20°C. Stock solutions were diluted to the desired concentrations with growth medium prior to use.

Plasmid construction and retroviral delivery

The DNA oligonucleotides used to generate shRNA against the open reading frame of Aur A were 5'-GUCUUGUGUCCUCAAUU-3'. pBabe/U6/puromycin/Aur A shRNA was generated according to the previously reported method [16]. The control vector was similarly constructed by directly inserting oligonucleotides encoding small hairpin RNA against green fluorescence protein (GFP) mRNA into pBabe/U6/puromycin. Retroviruses expressing Aur A shRNA or GFP shRNA were produced by transfection of pBabe/U6/shAur A or pBabe/U6/shGFP into Phoenix amphotropic cells and used to infect target cells (ME-180 cell line) by using a method described before [16]. Briefly, ME180 cells were infected twice for a total of 6 days (3 days for each infection) and the positive clones were selected with puromycin (200 ng/mL) for 10-14 days. The resulting cell lines were named ME180/shGFP and ME180/shAur A.

The plasmid used to enhance Aur A expression was constructed by inserting Aur A cDNA into pBabe/puromycin vector. The empty vector was used as control. Virus preparation and infection were the same as mentioned before [16]. The cervical cancer cell line SiHa was used for stable transfection of Aur A cDNA, and the established cell lines were named SiHa/Vector and SiHa/Aur A.

CCK-8 assays for cell proliferation

Cells were detached by trypsinization and washed twice with 1 × PBS. Then 2 × 10³ cells per well were seeded in 96-well culture plates (Corning Inc., Corning, NY) with 200 µL medium. After incubation for 2, 4, 6, 8, 10 days, the supernatant was removed, and cell viability was detected using Cell Counting Kit-8 (CCK-8) (Dojindo Laboratories, Kumamoto, Japan) according to the manufacturer's instructions. Absorbance at 450 nm was measured

using a microplate reader. The proliferation assays were performed independently and repeated at least 3 times.

IC₅₀ measurement

5×10^3 cells were seeded in 96-well cell culture plates and incubated overnight. Then the cells were exposed to different reagents for indicated days. Cell viability was detected using CCK-8 kit (Dojindo Laboratories, Kumamoto, Japan). IC₅₀ was calculated by the algebraic formula of the improved Karber method ($\lg IC_{50} = X_m - I [P - (3 - P_m - P_n)/4]$; X_m: lg (maximum dose), I: lg (maximum dose / adjacent dose), P: sum of the positive reaction rates, P_m: maximum positive reaction rate, P_n: minimum positive reaction rate. The assays were performed independently and repeated at least 3 times.

Examination of cell cycle and cell apoptosis

For cell cycle assay, cells ($1-2 \times 10^6$ per sample) were harvested and stained with PI and RNase as described before [16]. PI and RNase were purchased from Sigma-Aldrich (Missouri, US). Samples were determined by the FAC Station (Beckman Coulter, FV500) and analyzed by using CellQuest software. The assay was repeated three times.

To detect apoptosis, 1×10^5 cells were stained with Annexin V and propidium iodide supplied in the FITC Annexin V Apoptosis Detection Kit I (BD Biosciences, USA) and subject to analysis with a FAC Station (Beckman Coulter, FV500) equipped with CellQuest software. The experiment was done in duplicate and repeated three times.

Anchorage-independent colony formation

Soft agar assay was carried out according to our previous study [16]. The number of colonies > 50 µm (~100 cells) in diameter in each dish was counted at 14 to 20 days. The assay was repeated three times in duplicate.

Immunofluorescence

Immunofluorescence staining was done according to a published protocol [16]. DNA dye DAPI and primary antibody against Aur A were purchased from Molecular Probes (Eugene, OR). The secondary antibody Texas red-conjugated against rabbit IgG was obtained from Jackson

ImmunoResearch Laboratory (West Grove, PA). The stained cells were examined and photographed with a Leica SP5 confocal fluorescence microscope.

Immunoblot analysis

The preparation of whole-cell protein lysates and immunoblot analysis were performed as previously described [17]. Primary antibodies against Aur A, Bax, Bcl-2, CDK 2, CDK4, CDK6, cyclin E, cyclin D, cyclin A, p53, p21, Caspase 3, Caspase 7, Caspase 8 and Caspase 10 were obtained from Santa Cruz Biotechnology (CA, USA). Primary antibodies against β-actin, Bak, Bcl-x_s were purchased from Cell Signaling Technology (Danvers, MA). Donkey anti-mouse immunoglobulin (product NA931) and donkey anti-rabbit immunoglobulin (product NA9340) linked to horseradish peroxidase were from Amersham Biosciences (Little Chalfont, Buckinghamshire, UK). Immunoblot reagents were supplied in an electrochemiluminescence kit (Millipore, Bedford, MA, USA).

Xenograft tumors in nude mice

Animal assays were carried out with the institutional guidelines and were approved by the Institutional Animal Care and Use Committee of Fudan University Shanghai Cancer Center. Mice were kept in a pathogen-free environment. To generate xenograft tumors, we subcutaneously injected SiHA/Aur A, ME180/shAur A and corresponding control cells into 4- to 6-week-old BALB/c athymic nude mice. Briefly, 5×10^6 cells were harvested by trypsinization and washed twice with $1 \times$ PBS, then resuspended in $150 \mu\text{L } 1 \times$ PBS, and injected subcutaneously into 4- to 6-week-old BALB/c athymic nude mice (Department of Laboratory Animal, Fudan University). Six mice were used per cell line (SiHA/Aur A and ME180/shAur A, and their control cells SiHa/vector and ME180/shGFP) and each mouse received two injections, each of 5×10^6 cells in bilateral flank to form two tumors per mouse. The tumor growth of modified and control cell lines was monitored until the day that mice were killed. The date on which the first grossly visible tumor appeared for subcutaneous injection was recorded, and the tumor size was measured every 5 days. Two-dimensional measurements were taken with an electronic caliper after injection, and tumor volume was calculated with the use of the follow-

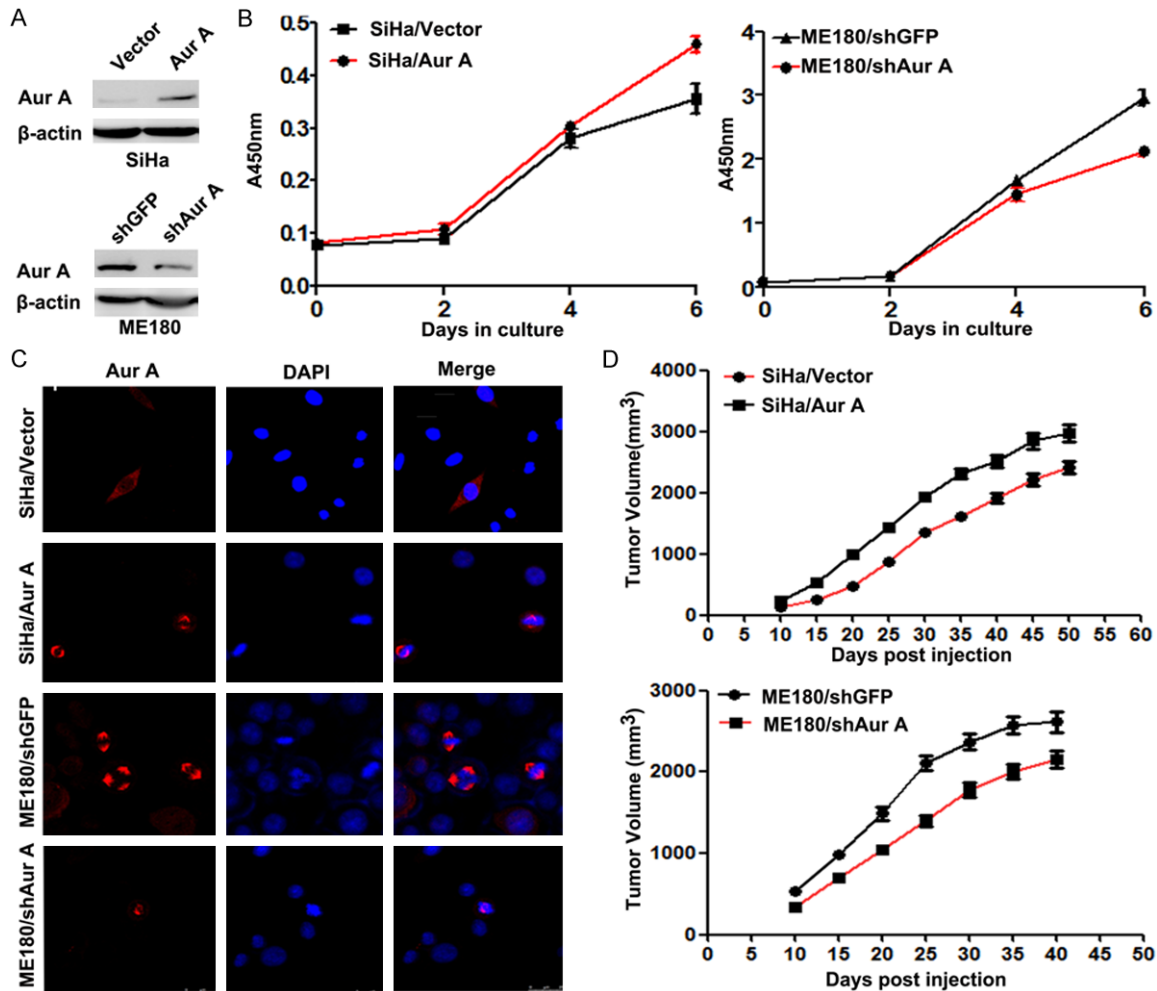


Figure 1. Aur A promotes tumorigenesis *in vitro* and *in vivo*. A. Detection of Aur A by immunoblot in SiHa/Vector, SiHa/Aur A, ME180/shGFP and ME180/shAur A cell lines. B. Examination of growth rates of SiHa/Vector, SiHa/Aur A, ME180/shGFP and ME180/shAur A cells. C. Analysis of spindle formation in SiHa/Vector, SiHa/Aur A, ME180/shGFP and ME180/shAur A. D. Tumor formation *in vivo*.

ing formula: tumor volume (in mm³) = $a \times b^2 \times 0.52$, a refers to the longest diameter, b is the shortest diameter, and 0.52 is a constant to calculate the volume of an ellipsoid. When a tumor reached 1.0 cm in diameter, the mouse was killed by exposure to 5% carbon monoxide.

Immunohistochemical staining and analysis

Tumor tissue samples were from Fudan University Shanghai Cancer Center and used following the written consent with patients and the institutional guidelines approved by the Institutional Committee of Ethics, Fudan University Shanghai. The tissue sections were treated with primary antibodies according to a previously published method [16]. The primary

antibody against Aur A (sc-25425, polyclonal antibody, Santa Cruz) or pERK1/2 (SC-23759, polyclonal antibody, Santa Cruz) was applied with the dilution of 1:100 at 4°C in a humid chamber. Tissue staining was performed by using DAKO Envision kit (DAKO, Carpinteria, CA) according to the manufacturer's instructions. Slides were then counterstained with aqueous hematoxylin (Shandon, Pittsburgh, USA) and mounted with a crystal mount. The test that the primary antibody was replaced with 1 × PBS was used as a negative control.

Evaluation of staining intensity for Aur A and pERK1/2 was independently done by two pathologists (Bin Chang and Gong Yang) in a blinded manner by using a scoring system based on both percentage of positive tumor

Table 1. Statistic analysis of cells under mitosis

	SiHa/ Vector	SiHa/ Aur A	ME180/ shGFP	ME180/ shAur A
Cells under mitosis	5	16	21	9
Total cells	496	580	720	691
<i>P</i> value	< 0.05		< 0.05	

cells and staining intensity. The expression correlation between Aur A and pERK1/2 was analyzed from cases with scores for both Aur A and pERK1/2 staining.

Statistical analysis

The relationships between Aur A and pERK1/2 and each of the clinic-pathological parameters were analyzed by χ^2 -test. Other values were expressed as means \pm standard error of the mean (SEM). Statistical analysis was performed using SPSS and GraphPad Prism. *P* value less than 0.05 was considered statistically significant.

Results

Aur A promotes cell proliferation and tumorigenesis

To study the function of Aur A in human cervical cancer cells, we first examined the expression level of Aur A in two cervical cancer cell lines: SiHa and ME180. According to our results, the level of Aur A was low and nearly undetectable in SiHa, while Aur A was relatively high in ME180. Next we stably transfected Aur A cDNA into SiHa to enhance Aur A level and specifically abrogated Aur A expression in ME180 cells using shRNA. The expression of Aur A in the resulting cells was measured by immunoblot assay. As shown in **Figure 1A**, compared with control cells, Aur A was markedly increased in SiHa/Aur A cells, while in ME180/shAur A cells, Aur A was obviously inhibited, indicating that the delivery of Aur A cDNA and shRNA was quite successful and these cells could be used in the following experiments.

To investigate the role of Aur A during cervical cancer development, we performed assays to detect the effect of Aur A on cell proliferation. The results showed that the overexpression of Aur A significantly promoted cell proliferation while the knockdown of Aur A remarkably inhibited cell growth (**Figure 1B**).

Aur A is known to regulate bipolar spindle formation and chromosome segregation to promote mitosis [18]. We examined the status of centrosomes by immunofluorescent staining of Aur A in cervical cancer cell lines with different Aur A levels. As expected, the overexpression of Aur A increased the number of centrosome and disrupted the formation of normal polar mitotic spindles, while suppression of Aur A led to the formation of monopolar mitotic spindles (**Figure 1C**). We also counted the number of mitotic cells and analyzed statistically (**Table 1**). These results indicated that Aur A is a pivotal regulator in mitosis.

In addition, the xenograft tumor in nude mice showed that Aur A promoted tumor formation *in vivo*, which was consistent with the *in vitro* results (**Figure 1D**). These data suggested that Aur A exerted a pro-proliferation function both *in vitro* and *in vivo*.

Aur A regulates cell cycle and apoptosis in cervical cancer

To further study the biological function of Aur A in cervical cancer cells, we examined cell cycle and apoptosis by flow cytometry. The results showed that Aur A promoted cell cycle progression through enhanced G1-S transition (**Figure 2A**). The immunoblot data showed that several proteins directly participating in cell cycles, such as cyclins (cyclin E, D and A) and cyclin-dependent kinases (CDK2, 4, and 6) were upregulated in SiHa/Aur A cell line and inhibited in ME180/shAur A cell line when compared with control cell lines. During mitosis, the formation of an active cyclin D-CDK4/6 complex was the key step for quiescent cells to initiate cell cycle and cyclin E was required for S phase entry [19]. Besides, p21, an essential suppressor involved in the G1-S cell cycle transition [20], decreased in SiHa/Aur A cell line and significantly increased in ME180/shAur A cell line (**Figure 2B**). Those immunoblot data explained the mechanism for Aur A-induced G1/S transition.

Analysis of apoptosis using Annexin V and PI staining revealed that the overexpression of Aur A decreased apoptosis, while silencing of Aur A increased apoptosis (**Figure 2C**). The immunoblot results showed that Aur A regulated p53 level. In Aur A overexpression cell line (SiHa/Aur A), p53 was suppressed, while in Aur

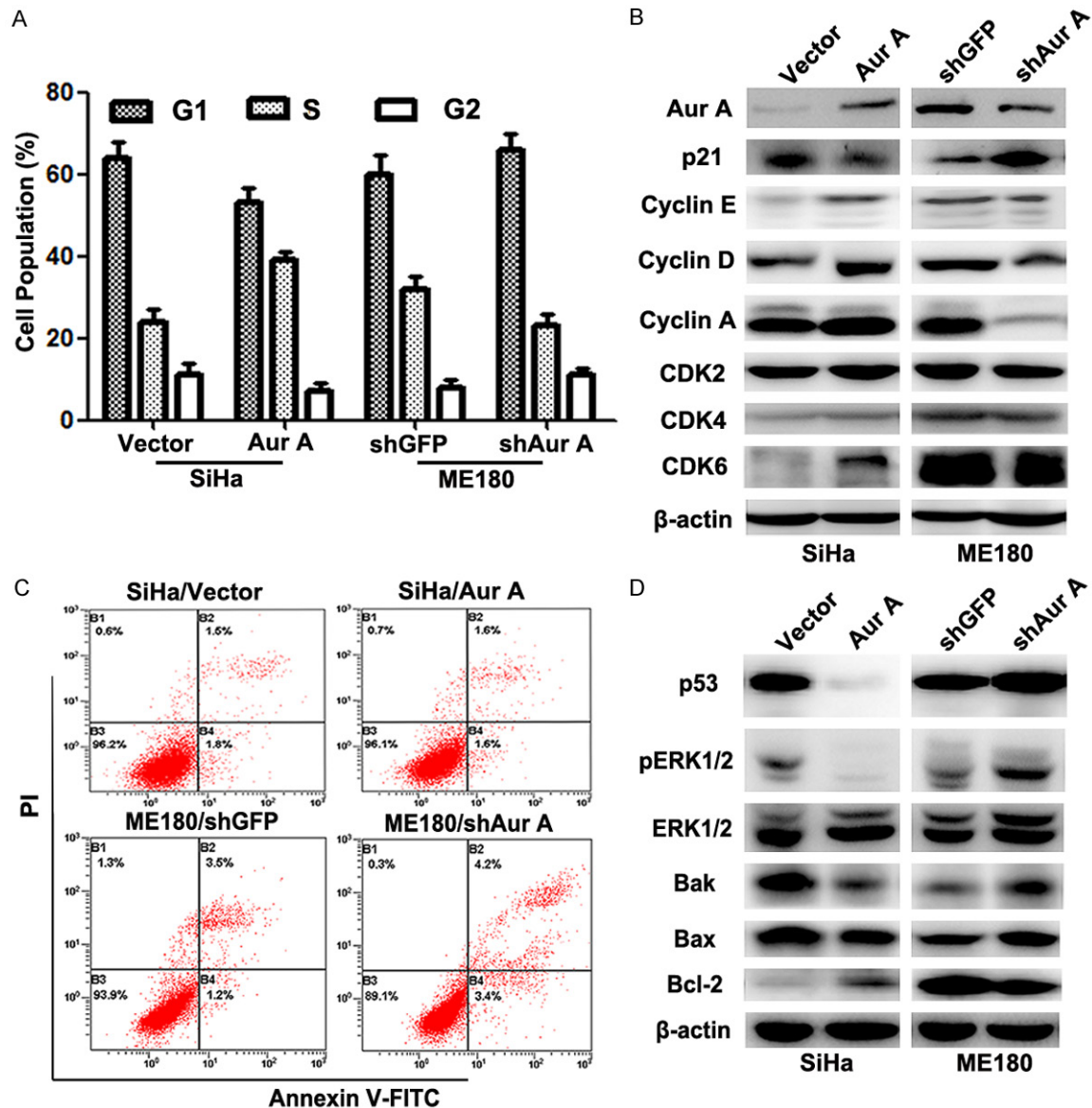


Figure 2. Aur A promotes cell cycle and protects cells from apoptosis. A. Detection of cell cycle by flow cytometry in SiHa/Vector, SiHa/Aur A, ME180/shGFP and ME180/shAur A cell lines. B. Analysis of cell cycle associated proteins by immunoblot in SiHa/Vector, SiHa/Aur A, ME180/shGFP and ME180/shAur A cell lines. C. Examination of apoptosis by flow cytometry in SiHa/Vector, SiHa/Aur A, ME180/shGFP and ME180/shAur A cell lines. D. Expression of Apoptosis-associated proteins detected by immunoblot in SiHa/Vector, SiHa/Aur A, ME180/shGFP and ME180/shAur A cell lines.

A knockout cell line (ME180/shAur A), p53 was maintained in a relative high level. As p53 is a main mediator in intrinsic apoptotic pathway, we next examined several apoptosis-associated proteins located in mitochondrial and found that in SiHa/Aur A cell line, the pro-apoptotic proteins Bak and Bax were decreased and the anti-apoptotic protein Bcl-2 was increased, while in ME180/shAur A cell line, Bak and Bax were upregulated and Bcl-2 was downregulated (Figure 2D). These results suggested that the

inhibition of Aur A promoted apoptosis through the intrinsic signaling pathway.

MAPK signaling is a major mediator in regulation of both cell cycle and apoptosis [21]. Next, we examined the status of the three members of MAPK including ERK, JNK, and p38, the results showed that Aur A overexpression in SiHa/Aur A cell line robustly inhibited the phosphorylation of ERK, while in Aur A knockdown cell line (ME180/shAur A), pERK1/2 was

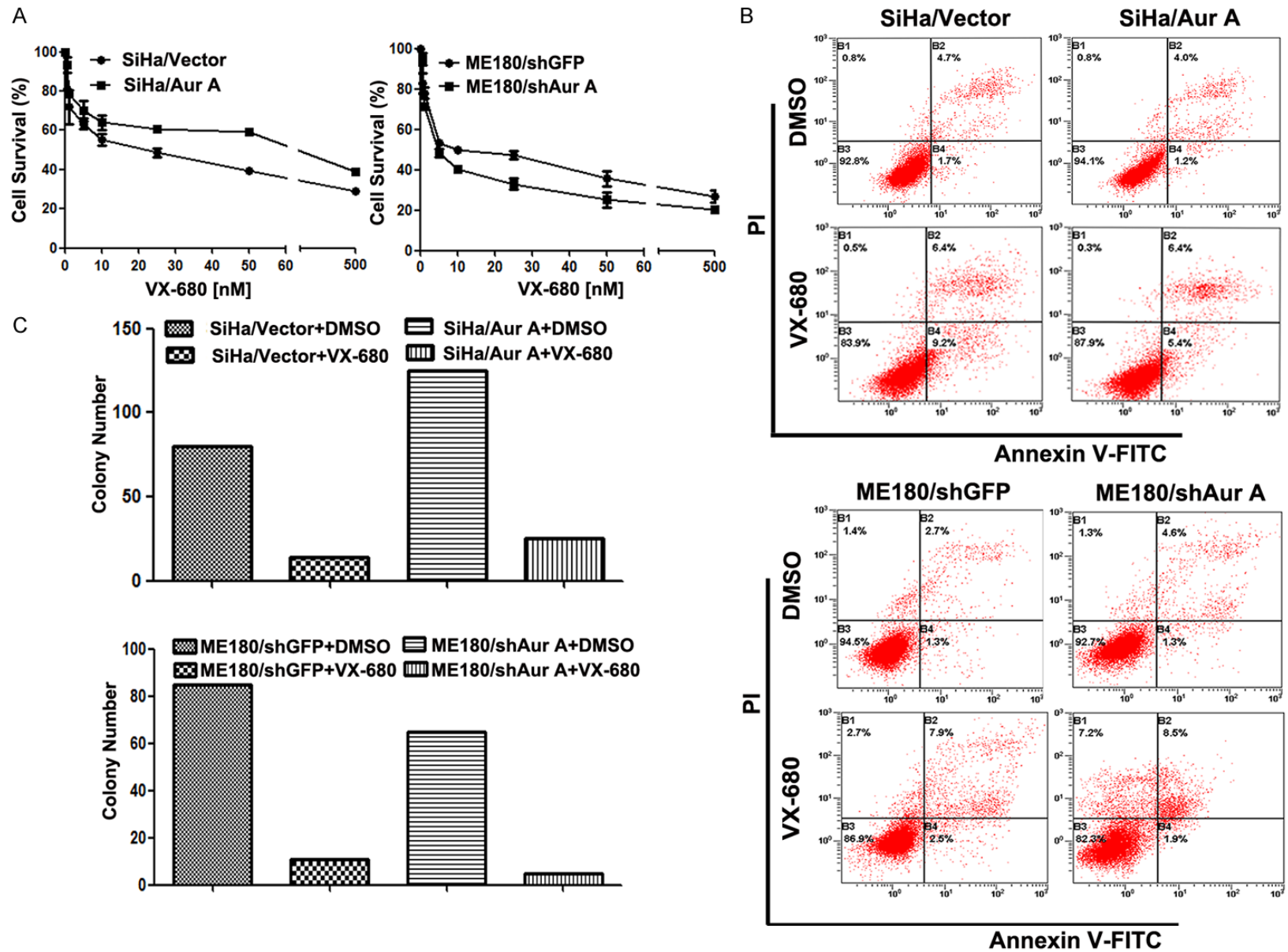


Figure 3. VX-680 blocks the oncogenic activity of Aur A. A. Cell viability after VX-680 treatment in SiHa/Vector, SiHa/Aur A, ME180/shGFP and ME180/shAur A cell lines. B. Cell apoptosis detected by flow cytometry after VX-680 treatment in SiHa/Vector, SiHa/Aur A, ME180/shGFP and ME180/shAur A cell lines. C. Colony formation after VX-680 treatment in SiHa/Vector, SiHa/Aur A, ME180/shGFP and ME180/shAur A cell lines.

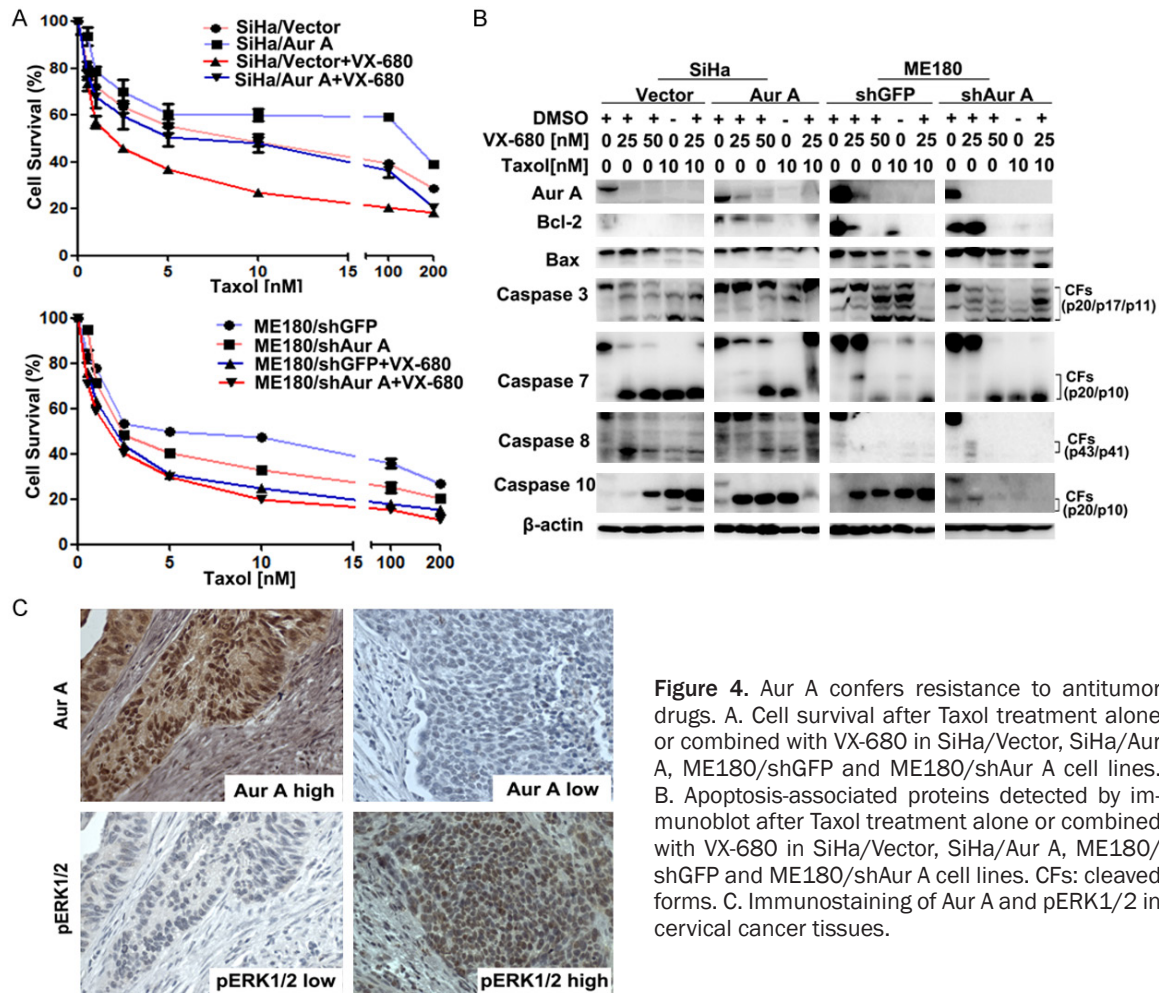


Figure 4. Aur A confers resistance to antitumor drugs. A. Cell survival after Taxol treatment alone or combined with VX-680 in SiHa/Vector, SiHa/Aur A, ME180/shGFP and ME180/shAur A cell lines. B. Apoptosis-associated proteins detected by immunoblot after Taxol treatment alone or combined with VX-680 in SiHa/Vector, SiHa/Aur A, ME180/shGFP and ME180/shAur A cell lines. CFs: cleaved forms. C. Immunostaining of Aur A and pERK1/2 in cervical cancer tissues.

enhanced (Figure 2D). However, no obvious changes of p-JNK and p-p38 were observed in both cell lines (data not shown). These data suggested that ERK might be involved in Aur A regulated cell cycle and apoptosis.

VX-680 improves the sensitivity of cervical cancer cells to Taxol

The development of small-molecule inhibitors for Aur A may make it possible to reduce or to block the oncogenic activity of Aur A, which thereby improves the survival of cancer patients [7, 22, 23]. To validate the potential effect of the Aur A inhibitor VX-680 on cervical cancer cells, we conducted assays to test the cell vi-

ability and apoptosis induced by VX-680. The results showed VX-680 decreased cell viability (Figure 3A), promoted apoptosis (Figure 3B) and inhibited colony formation (Figure 3C), suggesting that VX-680 inhibited the tumorigenic effect of Aur A.

It has been reported that overexpression of Aur A can overcome the spindle checkpoint induced by Taxol and confer cells chemoresistance [15]. Therefore, inhibition of the Aur A kinase activity may be a valuable adjunct to Taxol in the treatment of cancers overexpressing Aur A [24-26]. Then we conducted assays to test the cell viability by Taxol treatment alone, and subsequently by combination with VX-680. As shown

Table 2. Aurora-A overexpression according to clinic-pathologic characteristics of cervical carcinoma patients

	No. of patients	Aur A positive No (%)	P value	pERK1/2 positive. No (%)	P value
Age, years					
< 40	18	14 (77.78%)	P = 0.822	3 (16.67%)	P = 0.532
≥ 40	162	135 (83.33%)		20 (12.35%)	
Tumor size					
< 4 cm	68	41 (60.29%)	*P = 0.023	12 (17.65%)	*P = 0.037
≥ 4 cm	112	107 (95.53%)		6 (5.36%)	
Pathology					
SCC	152	121 (79.60%)	P = 0.903	30 (19.73%)	P = 0.742
AC	14	14 (100%)		2 (14.29%)	
ASC	14	14 (100%)		2 (14.29%)	
FIGO stage					
Ib	104	81 (77.88%)	P = 0.501	10 (9.62%)	P = 0.698
IIa	72	63 (87.5%)		5 (6.94%)	
IIb	4	4 (100%)		0 (0%)	
LVSI					
Positive	81	76 (93.83%)	*P < 0.001	7 (8.64%)	P = 0.212
Negative	99	72 (72.73%)		13 (13.13%)	
LN					
Positive	50	50 (100%)	*P < 0.001	2 (4%)	*P = 0.028
Negative	130	100 (76.92%)		17 (13.08%)	
Deep invasion					*P = 0.032
< 1/3	36	23 (63.89%)	*P = 0.014	6 (16.67%)	
1/3~2/3	54	36 (66.67%)		6 (11.11%)	
≥ 2/3	90	90 (100%)		3 (3.33%)	

SCC: Squamous cell carcinoma, AC: Adenocarcinoma, ASC: Adenosquamous carcinoma, FIGO: International Federation of Gynecology and Obstetrics, LVSI: Lympho-vascular space invasion, LN: Lymph node. *: significant difference ($P < 0.05$).

in **Figure 4A**, cells with high Aur A expression were more resistant to the chemotherapeutic agent Taxol and the inhibition of Aur A by VX-680 synergistically enhanced cell apoptosis to Taxol treatment. The results suggested that the treatment of cells with Aur A inhibitor in combination with Taxol could improve the therapeutic efficiency of Taxol. To further investigate the underlying molecular mechanism, we examined several major molecules involved in the intrinsic apoptotic pathway (Bcl-2, Bax) and the extrinsic apoptotic pathway (caspase 3, 7, 8, 10). As the results demonstrated in **Figure 4B**, in cell lines with relative low Aur A level (SiHa/vector and ME180/shAur A), the combined treatment induced the more remarkable changes than those treated with VX-680 or Taxol alone. In cell lines with high Aur A (SiHa/Aur A and ME180/shGFP), the combined effects were weaker than cell lines with low Aur A expression. These data validated the role of Aur

A in chemoresistance and provided a new insight in the combined treatment of cervical cancer.

Aur A and pERK1/2 are negatively correlated in cervical cancer tissues

To investigate the relationship between Aur A overexpression with clinical pathologic parameters including tumor size, cell type, FIGO stage, Lympho-vascular space invasion (LVSI), Lymph node metastasis (LN) and deep invasion, we analyzed the expression of Aur A in 180 cervical cancer tissues using IHC assay. Representative photomicrographs for Aur A are shown in **Figure 4C**. Results of immunostaining are summarized in **Table 2**. The results showed that Aur A was overexpressed in cervical cancer, and significantly correlated with tumor sizes ($P = 0.023$), LVSI ($P < 0.001$), LN ($P < 0.001$) and deep invasion ($P = 0.014$). Moreover,

we found that the level of Aur A was negatively correlated with pERK1/2 in cervical tissues ($P < 0.05$), as evidenced by the representative images showing that the high expression of Aur A was accompanied with the low level of pERK1/2 in same tissue blocks, while low expression of Aur A corresponded to high level of pERK1/2 (**Figure 4C**). Moreover the negative correlation was consistent with the above immunoblot results (**Figure 2D**). Therefore the combined analysis of Aur A and pERK1/2 may provide clues for cervical cancer diagnosis and treatment. Due to the limited time of follow-up visits, the correlation of Aur A and pERK1/2 expression with patient survival is uncertain. According to others' reports, patients with high Aur A expression predicted a poor disease-free survival and overall survival rates [27], suggesting the importance of Aur A in cervical cancer.

Discussion

Aur A belongs to a small family of serine/threonine kinases with evolutionarily conservative structure and participates in mitosis [28]. Aur A maintains a relatively high level in a wide range of cancer types via amplification or overexpression [29]. Accumulating evidence showed that Aur A plays a pivotal role in tumorigenesis [16, 28, 30, 31]. Previous studies indicated that Aur A is overexpressed in cervical carcinoma [30]. In clinic, the expression of Aur A is significantly higher in cervical carcinoma tissue than in normal tissue [32]. Patients with the high Aur A expression had a poorer disease-free survival and overall survival rates than patients with low Aur A expression, suggesting that the high Aur A expression is an independent prognostic factor in cervical cancer [27]. But there is a lack of literature on the biological function of Aur A in cervical cancer. In this paper, we investigated the role of Aur A in cervical cancer by delivering Aur A cDNA or shRNA into cells to establish stably transfected cell lines. Our data indicated that in cervical cancer cells, Aur A acts as an oncogene to stimulate cell proliferation both *in vitro* and *in vivo*, to promote cell cycle progression through the enhanced G1-S transition, to protect cells from apoptosis, to induce centrosome amplification, multipolar spindle formation and genomic instability, and consequently to confer resistance to antitumor agents. We further found VX-680, a specific inhibitor for

Aur A could improve the efficiency of Taxol in treatment of cervical cancer cells.

The fact that Aur A promotes tumorigenesis is reported to be mediated through different signaling pathway in multiple cell types. We showed that Aur A regulates cell cycle and apoptosis possibly through p53 and ERK. Because Aur A is reported to phosphorylate p53 at Ser³¹⁵ and enhances the affinity of p53 with Mdm2 to promote the degradation of p53 [33]. Besides, Aur A phosphorylates p53 at Ser²¹⁵ to abrogate the DNA-binding and trans-activation activity of p53 which subsequently regulates the expression of p53 downstream target genes, including p21 and Bcl-2 family proteins, such as Bak, Bax and Bcl-2 [34-37], which is consistent with what we found in cervical cancer cells. In addition, we demonstrated that ERK might be involved in Aur A-induced tumorigenesis. Although activation of ERK is generally considered to promote cell proliferation and survival, reports also exist that ERK transmits pro-apoptotic signals in several cell types [38, 39]. ERK has been shown to promote p53 accumulation, apoptosis and cell cycle arrest [40]. Moreover, ERK activity is associated with the upregulation of proapoptotic members of the Bcl-2 family, such as Bax [39]. In our study, we found that the level of Aur A expression and the phosphorylated status of ERK was negatively correlated, indicating that Aur A may inhibit the activation of ERK to promote cell cycle from apoptosis. We also examined the other two MAPK members, p38 and JNK, but there was no obvious differences in terms of Aur A overexpression or silencing.

Overexpression of Aur A has been shown to induce resistance to tubulin-targeted agents, such as Taxol by enhancing the mitotic spindle formation during mitosis [7]. Thus Aur A amplification may predict poor responsiveness to Taxol and other agents that target the spindle checkpoint [15]. RNA interference against Aur A suppresses tumor growth and enhances the sensitivity to chemotherapy-induced apoptosis in human cells, showing its potential as a target for cancer therapy [41]. Thus far, several inhibitors of Aurora kinases have been reported. VX-680, the first Aurora inhibitor used in clinical trials, functioning to disrupt mitosis, to inhibit proliferation, and to promote apoptosis, has been proven to be a high potent, selective, and

reversible inhibitor [14, 23, 42]. Although VX-680 is a pan-Aurora inhibitor, it has been proved to exert higher inhibitory activity against Aur A compared with Aur B and C especially under low concentration [42]. In our study, VX-680 indeed inhibits cell proliferation and enhances the sensitivity of cancer cells to chemotherapeutic agent Taxol by inducing both extrinsic and intrinsic apoptotic pathways.

In summary, these data have provided interesting evidence to reveal that overexpression of Aur A contributes to the resistance of anti-cancer drugs in cervical cancer cells and knock-down of Aur A may increase the sensitivity of Taxol-induced apoptosis in cervical cancer cells through multiple signal pathways.

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Disclosure of conflict of interest

The authors have no conflict of interest to declare.

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