Aurora-A, a Negative Prognostic Marker, Increases Migration and Decreases Radiosensitivity in Cancer Cells

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Abstract

Centrosomal Aurora-A (Aur-A) kinase ensures proper spindle assembly and accurate chromosome segregation in mitosis. Overexpression of Aur-A leads to centrosome amplification, aberrant spindle, and consequent genetic instability. In the present study, Aur-A was found to be overexpressed in laryngeal squamous cell carcinoma (LSCC). Moreover, Aur-A expression was adversely correlated with median survival, and further identified as a potential independent factor for disease prognosis. Suppression of Aurora kinase activity chemically or genetically led to LSCC Hep2 cell cycle arrest and apoptotic cell death. Importantly, we found that Aur-A increases cell migration and this novel function was correlated with Akt1 activation. The enhanced cell migration induced by Aur-A overexpression could be abrogated by either small-molecule Akt1 inhibitor or short interfering RNA. VX-680, a selective Aurora kinase inhibitor, decreased Akt1 phosphorylation at Ser473 and inhibited cell migration, but failed to do so in constitutive active Akt1 (myr-Akt1)–overexpressed cells. Moreover, our data suggested that overexpression of Aur-A kinase might also contribute to radiosensitivity of LSCC. Inhibiting Aur-A by VX-680 induced expression of p53 and potentely sensitized cells to radiotherapy, leading to significant cell death. Ectopic overexpression of Aur-A, however, reduced p53 level and rendered cells more resistant to irradiation. Taken together, we showed that Aur-A kinase, a negative prognostic marker, promotes migration and reduces radiosensitivity in laryngeal cancer cells. [Cancer Res 2007;67(21):10436–44]

Introduction

Head and neck squamous cell carcinoma (HNSCC), contributing to 6% to 10% of all cancers, is one of the most commonly occurring types of human tumor (1). Laryngeal squamous cell carcinoma (LSCC) accounts for the highest mortality among this group. LSCC remains the only type of cancer where no significant improvement has been made regarding 5-year survival rate in the past 25 years, making new therapeutic target identification an urgent task. Recent studies have shown that expressions of a number of tumor-associated genes are altered in LSCC, including EGFR (2, 3), p53 (4), p21 (5), and Bcl-2 (6). High-resolution cDNA array highlighted that alteration of gene expression may be critical to LSCC pathogenesis (7). However, few genes have provided consistent prognostic value for the disease, and the potential therapeutic significance of these genes remains to be clarified.

Mitotic serine/threonine kinases Aurora family, including Aur-A, Aur-B, and Aur-C, are key players in ensuring accurate chromosome segregation during the cell cycle, maintaining genetic integrity in cell division (8). Aur-C is highly expressed in sperm cells and has not shown a clear role in cancer (9). Aur-B phosphorylates histone H3 at Ser10 in vivo, dissociates HP1 from heterochromatin, and drives chromosome condensation (10). We and others previously showed that Aur-A was essential in proper timing of mitotic entry and formation of bipolar spindles (11, 12). Forced overexpression of Aur-A in cells leads to centrosome amplification and subsequent multipolar spindle structures (13, 14), causing genetic instability (aneuploidy), a hallmark of epithelial carcinoma (15, 16). Aur-A has been found overexpressed in various types of commonly occurring epithelial carcinomas (reviewed in ref. 17). Recent functional microarray analysis further identified Aur-A as one of the death-from-cancer signature genes differentially regulated in tumors (18). Small-molecule Aurora kinase inhibitors have been recently developed as potential targeting therapeutics (19). Among these, VX-680 with more Aur-A selectivity showed appealing preclinical evidence of anticancer activity in vivo (20–22).

Aurora kinases in tumorigenesis have been associated with their indispensable roles in ensuring proper mitotic events. Aur-A physically associates with a number of cell cycle regulators, including p53, TPX2, BRCA1, Ajuba, and TACC (15). For example, p53 binds to the NH2-terminal region of Aur-A and suppresses its oncogenic activity in vivo in a transactivation-independent manner (23). Conversely, Aur-A physically binds and phosphorylates p53 at Ser215 and Ser251 (24, 25). Phosphorylation at Ser215 destabilizes p53 and targets it for MDM2-mediated degradation. Consequently, elevated Aur-A expression impairs tumor-suppressing function of p53, and overrides apoptosis and cell cycle arrest induced by DNA damage. However, little is known about dysregulation of Aur-A kinase in other aspects of tumorigenesis besides its role in interrupting normal mitotic events.

Here, we found that Aur-A expression was elevated in LSCC tissues and Hep2 cells. Analysis of clinicopathologic characteristics revealed that Aur-A expression was adversely correlated with median survival. Cox regression analysis identified Aur-A as an independent factor for clinical prognosis. Suppression of Aur-A kinase activity by VX-680 or short interfering RNA (siRNA) inhibited cell growth and led to apoptotic cell death in Hep2 cells.
More importantly, we found that overexpression of Aur-A led to enhanced Akt1 phosphorylation at its activation site (Ser473) and remarkably increased cell migration ability. Suppression of Aur-A by either VX-680 or siRNA decreased Akt1 Ser473 phosphorylation and markedly reduced Hep2 cell migration rates. Moreover, in Hep2 cells with a constitutive active form of Akt1 (myr-Akt1), VX-680 failed to reduce cell migration. Last, we revealed that inhibition of Aur-A by VX-680 induced p53 expression and effectively sensitized laryngeal cancer cells to radiotherapy. Together, we showed that Aur-A served as both a potential negative prognostic biomarker for clinical prognosis and a promising molecular target for more selective therapeutic treatment in LSCC.

Materials and Methods

Patients and clinical tissue specimens. Patients were all first clinically diagnosed and pathologically confirmed of LSCC between 1995 and 1998. Of the participating patients, 15 patients had inadequate clinical follow-up, leaving 77 patients for analysis. Pertinent patient clinical reports were obtained with prior patient consent and the approval of the institutional Clinical Ethics Review Board. All of the 77 specimens and additional 35 normal adjacent tissues were collected and fixed in formalin and embedded in paraffin in the diagnostic histopathology laboratory at the Cancer Center of Sun Yat-sen University. A portion of tumor specimens were also kept in liquid nitrogen and sectioned for protein and RNA extraction. In all cases, the tissues were collected from the time of original diagnosis and before any treatments. Median clinical follow-up for the patients was 72 months (range from 3 to 136 months). Patient clinicopathologic features were shown in Supplementary Table S1. Tumors were staged according to Union Internationale Contra Cancrum classification (1997): stage I (14 cases), stage II (30 cases), stage III (15 cases), and stage IV (18 cases). Tumors were also classified as supraglottic, glottic, and subglottic, and were graded as well, moderately, and poorly differentiated.

Cell culture. Hep2 cells were obtained from American Type Culture Collection. Cells were cultured in RPMI 1640 (Invitrogen) supplemented with 10% fetal bovine serum (FBS; Hyclone), penicillin (100 units/mL), and streptomycin (100 units/mL) at 37°C in humidified 5% CO2 incubator.

Immunofluorescence staining. Cells were fixed in 2% paraformaldehyde (Electron Microscope Sciences) at room temperature for 20 min and permeabilized in 0.5% Triton X-100 in PBS for 10 min at 4°C. Immunostaining of cell was done as previously described (26) and visualized at 20× using a microscope (Leica).

Immunohistochemical staining of Aur-A expression. Tissue specimens in paraffin were sectioned, deparaffinized in xylene, and rehydrated. Antigenic retrieval was processed with sodium citrate. The sections were then incubated in H2O2 (3%) for 10 min, and blocked in 1% bovine serum albumin for 60 min followed by anti-Aur-A antibody (Upstate) at 4°C overnight. After being incubated with the secondary antibody for 60 min, specimens were incubated with H2O2-diaminobenzidine until the desired stain intensity developed. Sections were then counterstained with hematoxylin, dehydrated, and mounted. The extent of immunohistochemical staining of the sections was evaluated by at least two independent investigators. Moderate or strong cytoplasmic staining was considered as positive reaction. In analysis, specimen was determined as positive staining for Aur-A when >30% cells showed visible brown granules in the cytoplasm (27).

Reverse transcription-PCR. Cells or tissues were collected and immediately frozen in liquid nitrogen. Total RNA was extracted by using TRIzol reagent (Invitrogen). After reverse transcription of the total RNA, the first-strand cDNA was then used as templates for detecting of the Aur-A expression. A pair of primers, Aur-A-F: CATGGACCGATCTAAAGAAAACT and Aur-A-R: AAGATGGAGCATGTACTGACC, was used. For S26, the primers were S26-F: CCGTGGCCTCCAAAGATGACAAAG and S26-R: GTCGCCGCTCTTGCGGCTTCAC. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Cells were plated in 96-well flat-bottomed plates and exposed to DMSO or different doses of VX-680. Cell growth was assessed as described previously (28). Triplicate wells were assayed for each condition and SDs were determined.

Cell lysate and Western blot analysis. Cells or tissues were lysed on ice in radioimmunoprecipitation assay lysis buffer. The protein concentration was determined by the Bradford dye method (Bio-Rad Laboratories). Equal amounts of cell extract were subjected to electrophoresis in SDS-PAGE and transferred to nitrocellulose membrane (Bio-Rad Laboratories) for antibody blotting. Mouse anti–glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was from Ambion; rabbit anti–phosphorylated Aur-A/AIK (Thr288) antibody, mouse anti–cleaved poly(ADP)ribose polymerase (PARP) antibodies, mouse anti–phosphorylated histone H3 (Ser10), and rabbit anti–phosphorylated Akt (Ser473) were from Cell Signaling; goat anti-Akt1 was from Santa Cruz Biotechnology; and rabbit anti-Aurora A and mouse anti-p53 were from Upstate.

Flow cytometry analysis. Cells were seeded into six-well plates and incubated with VX-680 or DMSO at 37°C and collected. Single-cell suspensions were fixed in ice-cold 70% ethanol for 30 min, labeled with propidium iodide (50 µg/mL; Sigma-Aldrich) for at least 15 min in dark, and analyzed directly on a Becton Dickinson FACScan.

Transwell migration assay. Cells were starved overnight in assay medium (RPMI 1640 with 1% FBS). Top chambers of 24-well transwell plates (Corning, Inc.) were pretreated with 1% Matrigel (BD Biosciences) in PBS and incubated for 1 h at room temperature. Cells were added to the top chambers of the plates. Media with or without VX-680 were added to both chambers. After incubation, top cells were removed and bottom cells were fixed and stained with 4,6-diamidino-2-phenylindole (5 µg/mL) to visualize nuclei. The number of migrating cells in five fields was counted under fluorescence microscope, and the mean for each chamber was determined.

siRNA transfection. Cells were seeded onto six-well plate 16 h before transfection. In each well, 50 nM of siRNA Aur-A: AUGCCCUGUCUUA-CUGUCA, Akt1: AAGAGGGUGUGGCUGCAAAA, or scramble sequences and 10 µL of LipofectAMINE 2000 (Invitrogen) were added to Opti-MEM (Life Technologies) and mixed. After incubation, the siRNA and LipofectAMINE 2000 solutions were mixed gently and added to the plates. The plate was incubated for 48 h until it is ready for further assay.

Generation of stable transfection cell lines. pBabe-puro human Aur-A was constructed by inserting BanHI and XhoI fragment from pCS2+-Aur-A (a gift from Joan Ruderman, Harvard Medical School, Boston, MA) into BanHI and SalI sites. Myr-Akt1 was cloned by PCR from Myr-Akt1 (pUSEamp, Upstate) and digested with EcoRI and BanHI. The fragment was ligated into pBabe-puro vector. Vascular stomatitis virus (VSV) pseudotyped viruses were produced by transfection of the VSV-GP producer cell line (a gift from Joan Brugge, Harvard Medical School) with 10 µg of DNA using LipofectAMINE 2000 (Invitrogen). Hep2 cells were infected with the retroviral viruses, and stable populations were chosen by selection with 2 µg/mL puromycin (Sigma-Aldrich).

Radiation clonogenic survival assay. Two hundred cells were seeded onto six-well plate and incubated 24 h before irradiation. After attachment, cells were treated with VX-680 in some cases. Cells were then irradiated with 0 to 2 Gy of irradiation dose of X-ray. Cells were stained with Giemsa dye and clones were counted on day 10, and survival fractions were calculated to evaluate radiosensitivity.

Statistics. Survival was defined as months from diagnosis to death because of any cause. Survival was determined by the method of Kaplan and Meier. Differences of survival due to Aur-A expression were compared between groups of patients by the log-rank test. The effect of predictive variables was evaluated by Cox univariate and multivariate regression models. All P values quoted are two sided. P < 0.05 is considered statistically significant. Statistical analyses were done using SPSS v. 11.0 (SPSS, Inc.).

Results

Aur-A is overexpressed in LSCC tissues and cell line. To study the potential role of Aur-A in pathogenesis of LSCC, we first asked if the level of Aur-A protein was elevated in primary tumor tissues. Seventy-seven pathologically confirmed tumor specimens and 35

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matched adjacent normal tissues were collected for immunohistochemical analysis with Aur-A antibody. Aur-A was highly expressed in tumor specimens with a positive rate of 71.4% (55 of 77) when >30% of intracellular staining in a paraffin section was defined as positive (27). All adjacent normal tissues studied (35 of 35) were lower in Aur-A expression. A typical immunohistochemical staining of either specimen was shown in Fig. 1A. Notably, in each LSCC tissue, Aur-A was strongly stained within tumor foci of epithelial cells, whereas staining of the adjacent connective tissues was also much weaker for Aur-A expression.

We next verified Aur-A expression in paired freshly frozen tumor and normal adjacent biopsy samples with Western blot analysis. Aur-A was highly expressed in tumor samples collected but was under detectable level in paired normal adjacent tissues, comparing four typical pairs (Fig. 1B). LSCC Hep2 cell line also expressed a high Aur-A level. In addition, total RNA from tumor samples and their matched normal adjacent tissues were extracted and measured for Aur-A transcripts by reverse transcription-PCR (RT-PCR). Figure 1C showed two typical examples of increasing copies of Aur-A transcript in LSCC, whereas their normal adjacent tissues expressed low levels of Aur-A.

Comparison of Aur-A–positive staining rates in various clinical subgroups revealed a positive correlation between Aur-A expression and clinical tumor stage (Supplementary Table S1). Tumor level of Aur-A was significantly higher in high-grade (stages III and IV, 87.9%) versus low-grade (stages I and II, 59.1%) tumor (P = 0.006). We also observed a trend of preferential expression of Aur-A in poorly differentiated tumor cells (93.8%) compared with moderate (61.5%) or well-differentiated (68.6%) samples (P = 0.071). Aur-A expression was, however, not associated with age, sex, or clinical classification.

Aur-A expression is inversely correlated with survival rate in LSCC patients. Because tumor Aur-A level was associated with advanced tumor stage, we next asked if Aur-A was correlated with patient prognosis. The median follow-up for the 77 patients was 72 months (range, 3–136 months). As shown in Fig. 1D, overall median survival time clearly revealed that tumors with positive Aur-A staining were adversely corrected with patient prognosis, with 46 months (95% confidence interval, 19.5–72.5 months) for Aur-A–positive cancer patients versus 85 months (95% confidence interval, 66.8–103.2 months) for Aur-A–negative patients (P = 0.0159). Univariate analysis showed that tumor differentiation...
grade ($P = 0.004$), tumor stage ($P = 0.000$), clinical classification ($P = 0.023$), positive tumor staining at surgical margin ($P = 0.001$), and Aur-A ($P = 0.020$) each significantly predicted worse prognosis in LSCC patients (Supplementary Table S2). Among these prognostic factors, tumor stage, differentiation grade, and clinical classification has each been indicated to be associated with outcome of the disease (29). Clinical prognosis was, however, not associated with age, sex, or treatment modality. We next included these prognostic factors in Cox multivariate analysis to avoid the possible interfering interaction effects. Supplementary Table S3 showed that Aur-A status ($P = 0.020$), as well as differentiation grade ($P = 0.027$), tumor stage ($P = 0.001$), and positive tumor staining at surgical margin ($P = 0.023$) was each an independent prognostic factor for LSCC. Clinical classification, however, was not an independent predictor ($P = 0.839$). Thus, we showed that tumor Aur-A level, as an independent prognostic factor, was adversely associated with clinical prognosis in LSCC patients.

**Aurora kinase inhibitor VX-680 inhibits the growth of Hep2 cells and causes apoptotic cell death.** Association of tumor overexpression of Aur-A with poor clinical prognosis raises the possibility of inhibition of Aurora kinase to attenuate LSCC cancer cells. We first asked if small-molecule VX-680, a selective Aurora kinase inhibitor (21), could suppress the proliferation of Hep2 cells, where Aur-A is highly expressed. As expected, VX-680 inhibited Aur-A autophosphorylation at Thr288 within its activation loop (Fig. 2A, top). At low doses (1–5 nmol/L), VX-680 inactivated Aur-A kinase; at higher doses (10 nmol/L), Aur-B activity was also inhibited, as determined by phosphorylation of its specific in vivo substrate histone H3 at Ser10 (Fig. 2A, bottom). Figure 2B showed that inhibition of Aurora kinase by VX-680 suppressed growth rate of Hep2 cells in a dose-dependent manner by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. We next addressed if inhibition of Aur-A activity by VX-680 would lead to apoptosis and arrest cell cycle at mitosis. Western blot assays showed that VX-680 led to apoptotic cell death in both dose-dependent and time-dependent manners (Fig. 2C). An apoptotic marker, cleaved PARP, was readily detected at 12 h in cells treated with VX-680 (5 nmol/L). VX-680 induces cell cycle arrest and apoptosis. Hep2 cells were incubated with VX-680 and collected at time points as indicated. The cell cycle arrest and apoptosis were assessed by flow cytometry.

![Figure 2](image-url)

**Figure 2.** Aurora kinase inhibitor VX-680 decreases cell growth and causes apoptotic cell death in Hep2 cells. A, VX-680 inhibits autophosphorylation of Aur-A at Thr288. Hep2 cells were incubated with increasing amounts of VX-680 or DMSO for 24 h. Cell lysates were subjected to Western blot analysis with phosphorylated Aur-A antibody (top two panels). VX-680 inhibits histone H3 phosphorylation at Ser10. Hep2 cells were incubated with increasing amounts of VX-680 or DMSO for 24 h. Histone H3 phosphorylation at Ser10 was detected by immunofluorescence staining with phosphorylation histone H3-Ser10-specific antibody (bottom panel). B, VX-680 inhibits cell proliferation in a dose-dependent manner. Hep2 cells were incubated with increasing doses of VX-680 or DMSO for 24 h. Cell survival rate was measured by MTT assay. VX-680 induces Hep2 cell apoptosis in dose-dependent (C, top two panels) and time-dependent (C, bottom two panels) manners. Cells were collected, lysed, and subjected to Western blot analysis with PARP-specific antibody. D, VX-680 induces cell cycle arrest and apoptosis. Hep2 cells were incubated with VX-680 and collected at time points as indicated. The cell cycle arrest and apoptosis were assessed by flow cytometry.
Aur-A siRNA induces apoptosis; overexpression enhances survival against VX-680. We next genetically suppressed endogenous Aur-A by siRNA and studied cell cycle and apoptotic events. Aur-A protein was substantially reduced (>60%) at 48 h after siRNA transfection (Fig. 3A). Figure 3B showed that suppression of Aur-A markedly arrested cells at G2-M and induced apoptotic cell death, consistent with chemical inhibition of Aurora kinase activity (Fig. 2). Knowing that VX-680 also at higher doses inhibits other oncogene products, including Flt3 (21, 30), Abl (31), and Lck (21), these data pointed that the main target of VX-680 (5 nmol/L) inhibition in LSCC cells was Aurora kinase. We then ectopically

**Figure 3.** Aur-A siRNA causes apoptosis; overexpression of Aur-A alleviates VX-680–induced apoptosis. Aur-A expression in Hep2 cells was suppressed by siRNA. Hep2 cells transfected with scramble or siRNA targeting Aur-A. A, cells were lysed and subjected to Western blot analysis with PARP-specific antibody. RNAi, RNA interference. B, cells were also stained with propidium iodide, followed by flow cytometry analysis to detect cell cycle arrest and cell apoptosis. C, Hep2 cells were transfected with human Aur-A (Aur-A) and pBabe (Vector). Aur-A expression level was detected by RT-PCR (top two panels) and Western blot (bottom two panels). D, Aur-A overexpressed and vector cell lines were incubated with VX-680 for 24 h. Cells were rendered to Western blot analysis with PARP-specific antibody.

**Figure 4.** VX-680 reduces laryngeal cancer cell migration. Hep2 cells were seeded for transwell migration assay in the presence of DMSO or increasing doses of VX-680. After 24-h incubation, migration rates were quantified by counting the migrated cells in five random fields. Original magnification, ×100. Data summarize three independent experiments (B). One representative of three independent experiments was shown (A). C, Hep2 cells were incubated with increasing doses of VX-680 for 24 h. Cell lysates were subjected to Western blot analysis with phosphorylated Akt Ser473 antibody.
overexpressed wild-type Aur-A in Hep2 cells. RT-PCR and Western blot analysis showed that both Aur-A transcripts and protein level were increased in these cells (Fig. 3C), rendering more resistance to VX-680–induced apoptotic cell death (Fig. 3D).

**Inactivation of Aurora kinase inhibits cell migration.** We next asked if small-molecule inactivation of Aurora kinase would attenuate Hep2 laryngeal carcinoma cell migration. Transwell migration assay revealed that VX-680 potently inhibited migration capacity of Hep2 cells in a dose-dependent manner (Fig. 4). As shown in Fig. 2A, at dose of 1 nmol/L, VX-680 potently inhibited Aur-A activity whereas Aur-B was not significantly affected, as assessed by phosphorylation of histone H3 at Ser10. Notably, VX-680 (1 nmol/L) effectively prevented nearly 80% of Hep2 cells from migrating through the semimembrane, suggesting an important novel role of Aur-A kinase in cell migration.

We then genetically suppressed Aur-A expression and tested if similar results were generated as chemical inhibition. Inhibition of Aur-A expression with siRNA reduced the migration capacity of Hep2 cells (Supplementary Fig. S1A). Conversely, ectopically introducing Aur-A in these cells markedly increased migration capacity (Supplementary Fig. S1B). Thus, it is conceivable that up-regulation of Aur-A in LSCC may contribute to cancer invasiveness.

**Aur-A promotes Hep2 cell migration by activation of Akt1.** To investigate whether Aur-A–regulated Hep2 migration might be mediated by Akt1, we detected Akt1 phosphorylation at Ser473, which correlates with Akt1 activation (32), in either Aur-A overexpressed or suppressed Hep2 cells. We found that Akt1 phosphorylation at this activation site was reduced in Aur-A suppressed cells but increased in cells overexpressing Aur-A (Fig. 5A). Similarly, Akt1 activation was also effected by chemical inhibition of Aur-A by VX-680 in a dose-dependent manner (Fig. 4C). Phosphorylated Akt1 (Ser473) expression was reduced as VX-680 dose increased. As shown by previous tests, VX-680 does not directly inhibit Akt1 (Ki > 1,000; ref. 21). Moreover, API-2, a specific inhibitor of Akt1, abrogated cell migration increased by Aur-A overexpression (Fig. 5B). Suppression of Akt1 by siRNA led to similar results (Fig. 5C). Last, we ectopically introduced a constitutive active form of Akt1, myr-Akt1, and found surprisingly that VX-680 did not lead to the similar migration inhibitory effects in these myr-Akt1–transfected cells (Fig. 5D). These data showed that Aur-A enhanced cell migration in Hep2 squamous cells, and further suggested that this function of Aur-A was mediated by Akt1 activation.

**Overexpression of Aur-A induces radioresistance; VX-680 sensitizes Hep2 cells for radiotherapy.** We next asked if radiosensitivity in LSCC cells would be decreased by transfection with Aur-A. As shown in Fig. 6A, Hep2 cells transfected with Aur-A were rendered more resistance to X-ray irradiation (0.5–2 Gy) than
control cells. Western blot analysis revealed that p53 protein level was reduced in Aur-A–transfected cells (Fig. 6B, top panels), consistent with previous report that Aur-A down-regulated p53 by targeting for MDM2 degradation (24). We then tested if chemical inhibition of Aurora kinase was able to enhance the sensitivity of Hep2 cells to radiotherapy. We found that VX-680 (1 and 2 nmol/L) inhibition of Aur-A kinase effectively sensitized LSCC cells to X-ray irradiation (0.5 Gy; Fig. 6C). Figure 6D showed a clear dose-dependent augment of p53 protein level in Hep2 cells treated with VX-680. Similar increase of p53 was also seen in Aur-A siRNA cells (Fig. 6B, bottom panels). These data suggested that Aur-A might be a critical regulator in radioresistance in LSCC cells; inhibition of Aurora kinase up-regulated p53 and sensitized cells to radiotherapy.

Discussion

Mitotic Aurora kinases are crucial in maintaining accurate chromosome segregation. Abnormal expression of Aur-A has been reported in various cancer types, and in some cases is associated with poor prognosis. However, Aur-A in LSCC has not been studied, particularly its nonmitotic-related role in tumorigenesis. In the present study, we found that Aur-A was overexpressed in LSCC tumor cells (Fig. 1). Moreover, overexpression of Aur-A was associated with higher tumor stage (Supplementary Table S1). Indeed, high Aur-A level was inversely associated with overall survival of LSCC patients (Fig. 1D), as an independent prognostic factor (Supplementary Tables S2 and S3). Additionally, we showed that specific inhibition of Aurora kinase by small-molecule VX-680 or siRNA suppressed LSCC cell growth and induced apoptotic cell death, whereas overexpression of Aur-A rendered certain survival advantage (Figs. 2 and 3). Most importantly, we found that suppression of Aur-A kinase by VX-680 or siRNA both potently inhibited tumor cell migration rates (Fig. 4; Supplementary Fig. S1), suggesting a novel nonmitotic role of this mitotic kinase in metastasis of squamous cell carcinoma. Our data further suggested that Aurora kinase–regulated cell migration might be mediated by Akt1 activity (Fig. 5). As a major therapeutic approach in LSCC, radiotherapy benefits most patients with early-stage cancers while preserving laryngeal function. Aurora kinase inhibitor VX-680 up-regulated p53 and effectively sensitized tumor cells to radiotherapy whereas excess Aur-A generated radioresistance in Hep2 cells (Fig. 6). These data have provided clear evidence that the mitotic kinase Aur-A plays important roles in cell migration and radioresistance in LSCC cells. Collectively, our studies suggested that Aur-A kinase served as both a prognostic biomarker and a potential therapeutic target in LSCC.

LSCC remains as one of the tumors of little therapeutic improvements in the past two decades (1). Identifying new prognosis predictive markers for individualized treatment approaches will offer great promise in improving the current...
tumor distant metastasis in squamous cell carcinoma (33). In cellular migration via inhibiting Rho/ROCK signaling and thrombospindolin 1 (34, 35). Here, we showed that Aurora kinase promoted laryngeal squamous cancer cells to migrate. This is of particular interest because a recent report showed that up-regulation of mRNA of Aur-A was significantly associated with tumor distant metastasis in squamous cell carcinoma (33). In searching for the possible mechanism for this new function of Aur-A, we found that Aur-A activity was positively correlated with Akt1 phosphorylation at its activation site Ser473 (Fig. 5). Up-regulation of Akt1 kinase has been shown to involve in altered cell migration status: Overexpression of Akt1 promotes migratory ability in cultured squamous carcinoma cells (36), pancreatic cancer cells (37), and fibrosarcoma cells (38); however, Akt1 reduces cell invasiveness in some cultured mammary cells (39, 40). Our data showed that inhibiting Akt1 by either its chemical inhibitor API-2 or siRNA abrogated Aur-A-enhanced cell migration, implying that Akt1 might act as a downstream mediator in Aur-A migration pathway. This Aur-A-Akt1 connection was further supported by the finding that transfection with myr-Akt1, an Akt1 constitutively active form, blocked VX-680–reduced cell migration (Fig. 5). Thus, our study showed an unrevealed role of Aur-A in enhancing cell movement, and this new function of Aur-A was associated with Akt1 activation in LSCC cells.

Radiotherapy is the major treatment modality for LSCC at early stages, particularly T1 and T2 cancers for organ preservation. Sensitivity of tumor cells to radiotherapy is critical for the probability of local control of the disease. We showed that Aur-A overexpression rendered radioresistance in LSCS cells (Fig. 6). Aur-A inhibition effectively increased the sensitivity of LSCC cells to radiotherapy. Moreover, Aur-A–regulated p53 expression was correlated with radioresistance. These data were consistent with the previous work that Aur-A down-regulated p53 function (24). Further study is needed to identify the subgroup of patients who would benefit from chemical inhibition of Aurora kinase in the setting of radiotherapy.

Taken together, high tumor level of Aur-A seems to be inversely associated with survival in LSCC patients as an independent predicting factor. This finding suggests that Aur-A would serve as a potential biological marker to identify a subgroup of patients of poor prognosis for new treatment approaches. Aurora kinase–directed small-molecule inhibitor suppresses cell growth, induces apoptosis, inhibits cell migration, and sensitizes LSCC cells to radiotherapy, offering an opportunity for future target-guided therapy.

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