Inhibition of Aurora-A suppresses epithelial–mesenchymal transition and invasion by downregulating MAPK in nasopharyngeal carcinoma cells

Xiang-Bo Wan1,2,†, Zi-Jie Long1,†, Min Yan1,†, Jie Xu1, Liang-Ping Xia1, Li Liu1, Yan Zhao1, Xue-Fei Huang1, Xian-Ren Wang1, Xiao-Feng Zhu1, Ming-Huang Hong2 and Quentin Liu1,†

1 State Key Laboratory of Oncology in Southern China, Department of Experimental Research and 2 Department of Nasopharyngeal Carcinoma, Cancer Center, Sun Yat-sen University, 651 Dongfeng Road East, Guangzhou 510060, China

†To whom correspondence should be addressed. Tel: +86 20 87343148; Fax: +86 20 87343171; Email: liuqlab@yahoo.com

Materials and methods

Patients and clinical tissue specimens

Patients were all newly diagnosed and pathologically confirmed as NPC between August 2004 and December 2005. None of the participating patients received any previous treatments. Among the participating patients, 11 patients were deficient of adequate clinical data, leaving 82 patients for analysis. The mean age of these patients was 45.5 years. Pertinent patient clinical reports were obtained with prior patient consent and the approval of the Institutional Ethics Review Board at Sun Yat-sen University. All the 82 specimens and additional 27 normal adjacent tissues were collected and fixed in formalin and embedded in paraffin in the diagnostic histopathology laboratory at the Cancer Center, Sun Yat-sen University. A portion of tumor specimens was kept in liquid nitrogen for protein and RNA extraction. Tumors were staged according to UICC classification (1997).

Semi-quantitative evaluation of immunohistochemical staining

Immunohistochemical staining was performed as described previously (19). The visible cellular brown granules were considered as high staining. Both staining intensity and extent were included to evaluate Aur-A expression. We graded the staining intensity as follows: negative (score 0), bordering (score 1), weak (score 2), moderate (score 3) and strong (score 4). Staining extent was

Abbreviations: Aur-A, Aurora-A; DAPl, 4′,6-diamidino-2-phenylindole; EMT, epithelial–mesenchymal transition; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; IKK, IκB kinase; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor-kappa B; NPC, nasopharyngeal carcinoma; siRNA, small-interfering RNA.

†These authors contributed equally to this work.
also grouped into five parts according to the percentage of high-staining cells in the field: negative (score 0), ≤25% (score 1), 26–50% (score 2), 51–75% (score 3) and 76–100% (score 4). The merged overall score >5 was regarded as high staining and those ≤4 were considered as low staining. The immunohistochemical staining was evaluated and scored by at least two independent pathologists.

Reverse transcription–polymerase chain reaction

Total RNA of NPC tissues, paired normal adjacent tissues and CNE-2 NPC cells were extracted utilizing TRIzol (Invitrogen, Carlsbad, CA). Expression of Aur-A and S26 was measured by reverse transcription–polymerase chain reaction with primers as described (19).

Cell culture

NPC cell lines, including poorly differentiated NPC cell lines (CNE-2 and SUNE-1) and well-differentiated NPC cell lines (CNE-1 and HK-1), were cultured in RPMI-1640 supplemented with 10% fetal bovine serum (Hyclone, Logan, UT) with appropriate antibiotics at 37°C in 5% CO2 humidified incubator.

Cell survival [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay

Cells were seeded into 96-well flat-bottom plates and exposed to increasing doses of VX-680 (Kava Technology, San Diego, CA). Standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay was performed (19).

Western blot analysis

CNE-2 cells or tissues were lysed on ice with lysis buffer. Equal amount of extract was loaded to electrophoresis in sodium dodecyl sulfate–polyacrylamide gel electrophoresis and then transferred to nitrocellulose membrane (Bio-Rad, Hercules, CA) for antibody blotting. Mouse anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH) antibody was obtained from Ambion (Austin, TX); rabbit anti-phospho-Aur-A/AIK (Thr288; pAur-A), mouse anti-cleaved poly (ADP-ribose) polymerase (PARP), rabbit anti-cleaved caspase-3 (Asp175), rabbit anti-Bcl-2, rat anti-Snail and mouse anti-phospho-histone H3 (Ser10; phistone H3) antibodies were from Cell Signaling Technology (Danvers, MA); rabbit anti-MAPK1/2, mouse anti-phospho-MAPK1/2 (Tyr202 and Tyr204; pMAPK1/2) and mouse anti-Bax antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA), and rabbit anti-Aur-A antibody was from Upstate (Lake Placid, NY).

Immunofluorescence staining

CNE-2 cells were fixed in 2% paraformaldehyde (electron microscope) for 20 min and permeabilized in 0.5% Triton X-100 in phosphate-buffered saline for 10 min at 4°C. Immunofluorescence staining of cell was performed as described previously (20) and visualized with fluorescence microscope (Olympus BX51). Mouse anti-E-cadherin antibody was from BD Biosciences (San Jose, CA) and rabbit anti-β-catenin was from Upstate.

Small-interfering RNA transfection

The small-interfering RNA (siRNA) target sequences used for CNE-2 cancer cells were ATGCCCTGTCTTACTGTCA for Aur-A and GACCGGATGT/C176 for MAPK1. Transfection of siRNA sequence was carried out as described (19).

Flow cytometry analysis

CNE-2 cells were seeded in six-well plates and treated with VX-680 (20 nM) or siRNA as well as 0.1% dimethyl sulfoxide or scramble sequence for 24 h. Single-cell suspensions were fixed in ice-cold 70% ethanol for 30 min, labeled with propidium iodide (50 μg/ml; Sigma, St Louis, MO) for 15 min in dark and analyzed on a Beckon Dickinson FACScan.

Generation of transfection cell lines

For stable transfection, wild-type Aur-A was introduced into CNE-2 cells as described (19). For transient transfection, MEK constitutive active form, MEK2DD plasmid (a gift from Dr Joan Brugge, Harvard Medical School) and kinase-dead Aur-A mutant (D274A, a gift from Dr Joan Ruderman, Harvard Medical School) were introduced into CNE-2 cells directly by lipofectamine 2000 (Invitrogen).

Invasion assay

Upper chambers of 24-well transwell plate (Corning Incorporated, Corning, NY) were coated with 50% Matrigel (BD Biosciences) in phosphate-buffered saline. CNE-2 and gene-transfected CNE-2 cells were incubated with or without VX-680 or U0126 (Sigma) for indicated time in the upper chamber. After incubation, invaded cells were fixed and nuclei were visualized with 4',6-diamidino-2-phenylindole (DAPI) (5 μg/ml). Invasion rate was quantified by counting the invaded cells in five random fields per chamber under the fluorescence microscope. Data summarized three independent experiments.

Statistical analysis

The correlation between Aur-A expression and each clinical features was evaluated by Fisher’s exact test and binary logistic regression model. All data were evaluated by Fisher’s exact test.

Aur-A promotes invasion in NPC

Table 1. Aur-A protein expression status in relation to clinical features in NPC patients (n = 82)

<table>
<thead>
<tr>
<th></th>
<th>High staining (n = 59; score &gt; 5)</th>
<th>Low staining (n = 23; score ≤ 4)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>47</td>
<td>16</td>
<td>0.386</td>
</tr>
<tr>
<td>Female</td>
<td>12</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥45.5</td>
<td>32</td>
<td>9</td>
<td>0.326</td>
</tr>
<tr>
<td>≤45.5</td>
<td>27</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Tumor stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1 and T2</td>
<td>12</td>
<td>11</td>
<td>0.026</td>
</tr>
<tr>
<td>T3 and T4</td>
<td>47</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Lymph node metastasis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N0</td>
<td>10</td>
<td>5</td>
<td>0.751</td>
</tr>
<tr>
<td>N0, N1 and N3</td>
<td>49</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Distant metastasis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M0</td>
<td>58</td>
<td>22</td>
<td>0.485</td>
</tr>
<tr>
<td>M1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Clinical stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I and II</td>
<td>10</td>
<td>11</td>
<td>0.10</td>
</tr>
<tr>
<td>III and IV</td>
<td>49</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Cranial bone invasion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>40</td>
<td>7</td>
<td>0.003</td>
</tr>
<tr>
<td>Negative</td>
<td>19</td>
<td>16</td>
<td></td>
</tr>
</tbody>
</table>

Fisher’s exact test.

Fig. 1. Aur-A expression in human NPC and normal adjacent tissues. (A) NPC tissues show high immunohistochemical staining of Aur-A. Clinical specimens were collected and examined by immunohistochemical staining with Aur-A antibody. Examples of Aur-A staining were presented. NPC tissue displayed high immunohistochemical staining (left panel), whereas normal adjacent tissue showed low staining (right panel; original magnification, ×200). Insets showed enlarged views (original magnification, ×400). (B) Aur-A is overexpressed in primary NPC tissues. Western blot analysis of Aur-A expression (upper panel) in NPC tumor (T), normal adjacent tissues (N) and NPC CNE-2 cells. Equal amounts of protein loading were determined by GAPDH. Total RNA extracted from NPC tumors (T), normal adjacent tissues (N) and CNE-2 cells was measured by reverse transcription–polymerase chain reaction (lower panel). S26 ribosomal RNA was used as an internal control.
Results

Overexpression of Aur-A is correlated with clinical stage and invasiveness in NPC

We used the immunohistochemical approach to analyze Aur-A expression in primary NPC specimens compared with paired normal nasopharyngeal tissues. Aur-A was highly expressed in NPC samples, particularly among the tumor-invaded zone, whereas normal paired tissues showed low immunohistochemical staining (Figure 1A). Consistently, both western blot and reverse transcription–polymerase chain reaction analyses revealed similar findings (Figure 1B). Moreover, poorly differentiated NPC-derived CNE-2 and well-differentiated NPC-derived CNE-1 and HK-1 cell lines displayed the similar high level of Aur-A (supplementary Figure 1 is available at Carcinogenesis Online). The clinical features (Table I) summarized that a significant proportion of NPC specimens (59/82, 72.0%) was of strong Aur-A staining (overall score 5–8), as calculated according to Materials and Methods. In contrast, only 1 in 27 samples (3.7%) showed high Aur-A staining in paired normal tissues. We further examined the relationship between Aur-A expression and NPC clinical characteristics. The Fisher’s exact test showed that Aur-A staining was correlated with tumor stage \((P = 0.026)\) and clinical stage \((P = 0.010)\). Importantly, Aur-A overexpression was highly correlated with cranial bone invasion \((P = 0.003)\). Furthermore, binary logistic regression model analysis confirmed that tumor stage, clinical stage and cranial bone invasion remained significantly correlated with Aur-A expression in NPC (Table II).

Aurora kinase small-molecule inhibitor VX-680 suppresses cell growth and induces apoptotic cell death in NPC cells

We next examined whether inhibition of Aur-A activity could suppress NPC cell growth. VX-680 (1 nM) inhibited Aur-A by reducing autophosphorylation at its activation site, Thr288 (Figure 2A). Aur-B activity was also suppressed at higher dose (5 nM), as determined by phosphorylation of its specific in vivo substrate histone H3 at Ser10.

### Table II. Aur-A overexpression is correlated with tumor stage, clinical stage and cranial bone invasion

<table>
<thead>
<tr>
<th></th>
<th>(\beta)</th>
<th>SE</th>
<th>Wald</th>
<th>Significance</th>
<th>Expectation ((\beta))</th>
<th>95% CI for expectation ((\beta))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor stage</td>
<td>−0.636</td>
<td>0.314</td>
<td>4.114</td>
<td>0.043</td>
<td>0.529</td>
<td>0.286–0.979</td>
</tr>
<tr>
<td>Clinical stage</td>
<td>−0.841</td>
<td>0.365</td>
<td>5.325</td>
<td>0.021</td>
<td>0.431</td>
<td>0.211–0.881</td>
</tr>
<tr>
<td>Cranial bone invasion</td>
<td>−1.222</td>
<td>0.592</td>
<td>4.266</td>
<td>0.008</td>
<td>0.294</td>
<td>0.092–0.940</td>
</tr>
</tbody>
</table>

Clinical data were analyzed with binary logistic regression model. \(\beta\), regression coefficient; SE, standard error; CI, confidence interval.
Consistently, suppression of Aur-A by small-interfering RNA did not affect histone H3 phosphorylation in CNE-2 cells (supplementary Figure 2 is available at Carcinogenesis Online). Inhibition of Aurora kinase by VX-680 suppressed CNE-2 cell growth (Figure 2B) and caused apoptosis (Figure 2C) in a dose-dependent manner. Similar findings were also observed in CNE-1 and SUNE-1 NPC cell lines (data not shown). The apoptotic indicators, cleaved PARP and cleaved caspase-3, were evidently detected in CNE-2 cells treated with VX-680 (20 nM). siRNA suppression of Aur-A expression led to similar findings (data not shown). Meanwhile, striking downregulation of Bcl-2 and upregulation of Bax, two key players in governing cytochrome c release from inner mitochondria membrane during apoptosis (21), were also demonstrated. Flow cytometry analysis revealed that inhibition of Aur-A by VX-680 led to cell cycle arrest at G2/M phase, similar to that seen in Aur-A siRNA-transfected cells (Figure 2D). These results suggested that suppression of cell growth was largely due to G2/M phase arrest and apoptotic cell death induced by VX-680 inhibition of Aur-A in NPC cells.

**Inhibition of Aur-A restores expression of epithelial markers and decreases cell invasion concomitantly with reduction of MAPK phosphorylation**

Our clinical analysis found that Aur-A overexpression was significantly correlated with NPC cranial bone invasion. We next asked if inactivation of Aur-A kinase would affect CNE-2 cells invasion. Transwell invasion assay revealed that VX-680 potently inhibited CNE-2 invasion in a dose-dependent manner (Figure 3A). VX-680, at dose of 1 nM when Aur-A was inhibited (Figure 2A), effectively prevented 65% of cells (P < 0.01) from crossing the Matrigel-coated transwell membrane pore, suggesting an important role of Aur-A in cancer cell invasion. EMT was a key initial step during tumor invasion (22). Western blot analysis and immunofluorescence staining showed that expression of epithelial membrane marker, E-cadherin, was increased markedly in cancer cells treated with increasing doses of VX-680. Furthermore, western blot analysis revealed that Snail, an E-cadherin transcriptional suppressor, was increased in Aur-A-overexpressed cells and was reduced by VX-680 treatment (supplementary Figure 3 is available at Carcinogenesis Online). Similarly, we also observed a nucleus to membrane translocation of β-catenin, suggesting a reversed EMT process. Interestingly, these membrane protein alternations occurred concomitantly with reduction of MAPK1/2 phosphorylation (Figure 3B). Additionally, suppression of Aur-A by siRNA or by transfected with kinase-dead Aur-A mutant (D274A) led to inhibition of MAPK1/2 phosphorylation and cell invasiveness (P < 0.001; Figure 4A and B). Conversely, ectopic overexpression of Aur-A enhanced MAPK1/2 phosphorylation (Figure 4Ca) and cell invasion (2-fold; P < 0.01; Figure 4Cb). Thus, our data indicated that Aur-A was involved in EMT-initiated invasiveness and correlated with activation of MAPK1/2 pathway.

**MAPK acts as the downstream component of Aur-A in inducing EMT and invasion in NPC**

We further studied whether MAPK pathway may relay Aur-A activity to promote EMT and invasion in NPC cells. We found that selective MEK kinase inhibitor U0126 (20 μM) completely abrogated cell invasion enhanced by Aur-A overexpression (P < 0.01; Figure 4B). Using siRNA, we showed that suppression of MAPK1 also markedly increased expression of epithelial membrane markers (Figure 5Aa). Moreover, MAPK1 siRNA (Figure 5Ab) blocked Aur-A overexpression-enhanced cell invasion (P < 0.01; Figure 5Ac). Importantly, we demonstrated that VX-680 failed to inhibit cell invasion in NPC cell transfected with a constitutive active form of MEK, MEK2DD (P > 0.05; Figure 5Ba and Bb). At the meantime, MEK2DD-transfected cells abrogated VX-680-elevated E-cadherin and β-catenin expression on cytoplasmic membrane (Figure 5Bc). These data suggested that MAPK acted as the downstream component of Aur-A in inducing EMT and invasion in nasopharyngeal cancer cells.
Discussion

The human homologues of Aurora kinases are important for the accurate execution of mitotic events and are essential for maintaining the genomic integrity. Aur-A played a significant role in ensuring the centrosome segregation and spindle assemble. The messenger RNA and protein level of Aur-A were commonly increased in various epithelial-derived malignant tumors (23–25). Recent studies in the physiological and pathological functions of the Aur-A have helped to elucidate its potential role in tumorigenesis (6,26). Our recent work has showed that Aur-A enhances migration of squamous carcinoma cells (19). Given the essential role of aberrant Aur-A expression played in tumor progression, inhibition of Aur-A offers an attractive approach for targeted cancer therapy. Recently, several inhibitors of Aurora kinases, including Hesperadin (27), ZM447439 (28), VX-680 (29), aurora kinase inhibitor (a ZM447439 synthetic intermediate) (30), MLN8054 (31) and AZD1152 (32), have been developed. Among which, Aur-A inhibitory VX-680 has displayed promising effects in inhibiting tumor growth in vivo, leading to potent and effective cytotoxicity in leukemia, prostate, colon and pancreatic cancers (20,29,33).

Fig. 4. Aur-A regulates membrane expression of epithelial markers and cell invasion via MAPK. (A) Suppression of Aur-A by siRNA restores epithelial markers expression and decreases cell invasion along with MAPK dephosphorylation. Aur-A siRNA or control (scramble) cells were subjected to western blot analysis with Aur-A, pMAPK1/2, MAPK1/2 and GAPDH antibodies (Aa), invasion assay (Ab) and immunofluorescence staining analysis (Ac; original magnification, ×1000). (B) Kinase-dead Aur-A-transfected cells decrease cell invasion and reduce MAPK phosphorylation. CNE-2 cells transfected with kinase-dead Aur-A mutant (pBabe-puro-Aur-A D274A) or vector (pBabe-puro) for 48 h were subjected to western blot analysis (Ba) and invasion assay (Bb). (C) Aur-A-overexpressed CNE-2 cells promote cell invasion and induce MAPK activation. CNE-2 cells were transfected with wild-type Aur-A (pCS2+-Aur-A) or vector (pCS2+) and subjected to western blot analysis (Ca). Aur-A-transfected cells were treated with or without U0126 (20 μM) prior to invasion assay (Cb).
Given the little therapeutic improvement of NPC in the past decades (17,18), uncovering new prognostic and therapeutic biomarkers became an urgent need for NPC treatment. Recent study in head and neck squamous cell carcinoma showed that both disease-free survival and shorter overall survival were strongly correlated with the Aur-A messenger RNA level (34), indicating that Aur-A may offer a prognostic biomarker in head and neck cancer (35). Moreover, upregulation of Aur-A messenger RNA and protein expression was significantly associated with occurrence of regional lymph node and distant metastasis. However, the relationship between malfunction of Aur-A and NPC tumorigenesis, particularly for tumor invasiveness, has not been studied. Here, we used clinical specimens and Aurora inhibitory VX-680 to address the possibility of Aur-A as a clinical biomarker and potential therapeutic target in NPC.

In this study, we found that Aur-A was overexpressed in NPC tissues, as assessed by immunohistochemical, western blot and reverse transcription–polymerase chain reaction analyses (Figure 1). Moreover, we found that Aur-A overexpression was correlated with tumor stage, clinical stage and cranial bone invasion rather than gender, age, lymph node metastasis and distant metastasis (Table I). Furthermore, poorly differentiated and well-differentiated NPC cell lines displayed the similar Aur-A expression, indicating that Aur-A expression was not related with NPC differentiation (supplementary Figure 1 is available at Carcinogenesis Online). Inhibition of Aur-A by its inhibitory VX-680 suppressed cell proliferation and induced mitochondria-related apoptotic cell death in CNE-2 (Figure 2) and other NPC cells (data not shown). VX-680 preferentially inhibits Aur-A (Ki = 0.6), along with its inhibitory effects on Aur-B (Ki = 18) and Aur-C (Ki = 4.6) (29). Indeed, VX-680 leads to G2/M arrest and monopolar spindle, characteristic of Aur-A defects, in a number of studies (19,20,36). VX-680 also inhibits other protein kinases, including Abelson tyrosine kinase (Ki = 68), lymphocyte-specific kinase (Ki = 80), fms-like tyrosine kinase 3 (Ki = 30) and MAPK (Ki > 1000), albeit with less potency (29,37). We showed that suppression of endogenous Aur-A by siRNA generated similar G2/M arrest and apoptotic cell death as seen in VX-680 treatment (Figure 2), indicating that antitumor effect of VX-680 was largely due to Aur-A inhibition in NPC cells.

EMT is a process whereby epithelial cells lose cell-to-cell adhesion characterized by repression of membrane proteins such as E-cadherin and β-catenin, and undergo dramatic cytoskeleton remodeling (38). The essence of EMT lies in disruption of intercellular contacts and
enhancement of cell motility, in turn leading to detachment of cells from the parental epithelial tissue, a prerequisite for tumor invasion (39). Interestingly, we found that Aur-A inhibition by VX-680 significantly suppressed the CNE-2 cell invasion ability, as well as reversed its EMT behavior by reducing membrane expression of epithelial markers E-cadherin and β-catenin (Figure 3). The possible involvement of Aur-A in NPC invasiveness was further supported by our clinical finding that overexpression of Aur-A was positively correlated with cranial bone invasion (Tables I and II). Interestingly, previous study reported that overexpression of Aur-A was a strong indicator for distant metastasis in head and neck squamous cell carcinoma (34). Our report is the first evidence that Aur-A, a well-characterized mitotic kinase, may also be involved in reorganizing cellular structures by regulating expression of cell-surface adhesion proteins, therefore promoting tumor invasion. Loss of membrane E-cadherin/β-catenin and gain of vimentin and N-cadherin have been considered as key events in EMT of squamous carcinoma cells. Transcriptional factors including Snail and Slug repress E-cadherin production at transcription levels (9,40,41). In the present work, we clearly demonstrated that inhibition of Aur-A upregulated membrane E-cadherin/β-catenin in NPC cells. Our initial results indicated that elevated E-cadherin expression by Aur-A inhibition was correlated with reduced level of Snail, an E-cadherin transcriptional repressor (supplementary Figure 3 is available at Cancerogenesis Online). Our ongoing study will further investigate the underlying mechanism of Aur-A and other EMT events.

Among various growth factor-activated signaling pathways (42–44), MAPK signaling is reported to contribute to EMT and invasiveness (45,46). For example, treatment with transforming growth factor-β in human mammary epithelial cells promoted EMT and invasion. Moreover, gene expression profiling revealed that these transforming growth factor-β-treated cells exhibited a specific 10-gene signature associated with MAPK signaling (47). Extended from previous reports that hyperactivation of MAPK acted as a critical component in the process of EMT and invasion, our work further identified that MAPK acted as a downstream component of Aur-A in promoting CNE-2 cells’ EMT and invasiveness (Figures 4 and 5). Previous study has identified a novel role of Aur-A as positive regulator of nuclear factor-kappa B (NF-kB) signaling (48). Suppression of Aur-A with VX-680 downregulated NF-kB activity in cancer cells (49). Indeed, Aur-A-mediated phosphorylation of IkBα at Ser27 and Ser36 was regulated by IkB kinase (IKK) (48). IKK was associated with cancer progression including invasion (50). Our data provided new insight into the role of Aur-A in tumor invasiveness via induction of MAPK pathway. The pathway of Aur-A leading to MAPK activation remains to be determined in future studies. However, IKK induced phosphorylation and degradation of NF-kB p105 (one member of IkB family) and subsequently released tumor progression locus 2 (TPL2) (MAP3K) from NF-kB p105. TPL2 in turn activated MAPK1/2 via phosphorylation of MEK1/2 (51). Thus, it is conceivable that IKK may also relay Aur-A signaling to induce MAPK activation. Intriguingly, Furukawa et al. (14) showed that Aur-A was one of the downstream targets of MAPK, which facilitated transcriptional factor V-ets erythroblastosis virus E26 oncogene homolog 2 (avian) binding to Aur-A promoter area and led to Aur-A overexpression in pancreatic cancer cells previously. Together with our findings, a positive feedback loop may exist between Aur-A and MAPK pathway in promoting cell invasion during tumorigenesis. Taken together, our study showed that elevated Aur-A expression was associated with tumor stage, clinical stage and cranial bone invasion in NPC. Most importantly, Aur-A enhanced EMT and invasiveness via activation of MAPK signaling pathway, offering an opportunity for future target-guided therapy. Aurora-directed small-molecule inhibitor VX-680 suppressed cell growth, induced apoptosis and decreased cell invasion, which may provide a promising molecular targeting agent in human NPC.

**Supplementary material**

Supplementary Figures 1–3 can be found at http://carcin.oxfordjournals.org/

### Funding

Sun Yat-sen University 985 Program Initiation Fund (to Q.L.); National Nature Science Foundation of China (30772476 to Q.L.); Guangzhou S & T Fund (031403 to Q.L.); Guangdong Medical Science Fund (B2008056 to M.Y.).

### Acknowledgements

We thank Li-Hui Wang, Fei-Meng Zheng and Jin-E Yao of Liu laboratory for their critical comments and technical support. We thank Guo-Wei Li (School of Public Health, Sun Yat-sen University) for his skillful assistance in data analysis and Min-Jie Chen (Olympus Company) for her technical support. We thank Dr Joan Brugge (Harvard Medical School) for kindly providing pBabe vector and MEK/2D plasmid. Special thanks to Dr Joan Ruderman (Harvard Medical School) for kindly providing Aur-A mutant (D274A) plasmid and support in general.

**Conflict of Interest Statement:** None declared.

### References

Aur-A promotes invasion in NPC


Received April 13, 2008; revised July 11, 2008; accepted July 25, 2008