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Aurora kinase small molecule inhibitor destroys mitotic spindle, suppresses cell growth, and induces apoptosis in oral squamous cancer cells

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Summary Mitotic Aurora kinases are required for accurate chromosome segregation during cell division. Ectopic expression of Aurora-A (Aur-A) kinase results in centrosome amplification, aberrant spindles, and consequent aneuploidy. In the present study, we showed that Aurora kinase inhibitory small molecule VX-680 inhibited histone H3 phosphorylation at Ser10, a known *in vivo* substrate residue of Aurora kinase, in oral squamous cell carcinoma (OSCC) KB cells. In addition, monopolar spindle structures, typical abnormalities induced by inhibition of Aur-A, were generated in VX-680-treated cells. Inhibition of Aurora kinase led to reduced KB cell growth, as assessed by MTT assay. Western blot analysis revealed that VX-680 caused cleavage of two critical apoptotic associated proteins, PARP and caspase-3. In contrast, expression of cell survival factor Bcl-2 was reduced by VX-680 treatment in a dose-dependent manner. Subsequently, nuclear characteristic of DNA fragmentation, indicative of apoptotic cell death, was clearly observed in these OSCC cells with Aurora kinase inhibitory VX-680. Taken together, we showed that Aurora kinase inhibitory VX-680 led to apoptotic cell death in OSCC cells, suggesting a novel therapeutic target in oral cancer.
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Abbreviations: Aur-A, Aurora-A; OSCC, oral squamous cell carcinoma; EGFR, epidermal growth factor receptor; BRCA1, breast cancer 1; TPX2, target protein for xklp2; TACC, transforming acidic coiled-coil; Flt3, Fms-like tyrosine kinase 3; Abl, Abelson tyrosine kinase

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Introduction

Oral squamous cell carcinoma (OSCC) ranks among the top ten most commonly occurring cancers worldwide. Despite the advances in diagnosis and therapy in the past two decades, OSCC still remains as one of the tumor types with poor prognosis.[1–3] Localregional relapse after therapy is a major cause of death in patients and has prompted substantial efforts in identifying new molecular targets that may lead to prolonged disease-free survival. Recent progress in molecular pathogenesis of this malignant disease has revealed genetic changes of a number of tumor associated genes, including epidermal growth factor receptor (EGFR),[4–8] Ras, cyclin D1,[9] p16,[10] and ERK1/2.[11] For example, EGFR expression is up-regulated in 80—90% of head and neck malignant lesions, including OSCC, and is generally associated with an adverse prognosis.[12] EGFR targeting regimens,[2,13,14] including monoclonal antibody, small molecule inhibitor, immunotoxin conjugates and anti-sense oligonucleotides, have been lately developed with promising results against head and neck malignancy.[15,16] Indeed, combination of radiotherapy with monoclonal antibody against the EGFR (cetuximab) resulted in significantly prolonged progression-free survival in locoregionally advanced squamous cell carcinoma of the head and neck, with the median duration of overall survival of 49 months among patients treated with combined therapy and 29.3 months among those treated with radiotherapy alone.[16]

Human centrosomal serine/threonine kinase Aurora family, including Aur-A, -B, and -C, govern accurate chromosome segregation during cell cycle, ensuring genetic stability in cell division.[17–20] Aurora kinase phosphorylates histone H3 at Ser10 *in vivo*, a key event in mitotic progression.[21] Aur-C, which is highly expressed in sperm cells, plays a crucial role in spermatogenesis.[22] Homozygous mutation of Aur-C generates polyploidy spermatozoa and causes male infertility.[23] Aur-B and -C both can associate with survivin and inner centromere protein (INCENP), and form chromosome passenger protein complex, controlling proper microtubule attachment to chromosomes and cytokinesis.[24–26] Aur-A is essential in proper timing of mitotic entry and formation of bipolar spindles,[27] and physically associates with a number of cell cycle regulators, including p53, target protein for xklp2 (TPX2), breast cancer 1 (BRCA1), Ajuba, and transforming acidic coiled-coil (TACC).[28] Abnormal interaction of Aur-A kinase with these proteins may disrupt well controlled cell cycle process, leading to tumorigenesis. Forced overexpression of Aur-A transformed NIH3T3 and immortalized Rat1 cells, and these transformed cells could form tumors when implanted in nude mice.[27,29,30]

Members of Aurora kinase family have been found overexpressed in various types of commonly occurring epithelial carcinomas.[29,31–34] Indeed, both Aur-A and -B have been found to be overexpressed in oral cancers.[35,36] In one study, Aur-A gene amplification was detected in 36% of oral cancers. Overexpression of this protein was however observed in all cases studied, suggesting a transcriptional up-regulation of Aur-A in tumorigenesis.[35] In addition, overexpression of Aur-A promotes Ras-induced oncogenic transformation. Recently, small molecule Aurora kinase inhibitors have been developed as potential targeting therapeutics.[37] Among these, VX-680 with more Aur-A selectivity showed appealing preclinical evidence of anti-cancer activity *in vivo*.[34,38,39] The potential anti-tumor function of VX-680 in OSCC, however, has not been studied previously.

Here we showed that Aurora kinase inhibitory VX-680 destructed normal mitotic spindles and generated monopolar structures in OSCC KB cells. Subsequently, cell growth was potently inhibited by VX-680, as assessed by MTT assay. Furthermore, VX-680 caused apoptotic cell death in KB oral cancer cells in a dose-dependent manner, producing apoptosis characteristic of DNA laddering fragmentation. Lastly, we showed that expression of Bcl-2 anti-apoptotic protein was suppressed and caspase-associated apoptosis pathway was activated in VX-680 treatment. In summary, we demonstrated that Aurora kinase inhibitory VX-680 decreased Bcl-2 expression and induced apoptosis in KB oral cancer cells, suggesting a potential molecular target for more selective therapeutic treatment in OSCC.

Materials and methods

Reagents and cell line

VX-680 was purchased from Kava Technology, San Diego, CA, stored at −20 °C at 400 μM in dimethylsulfoxide (DMSO), and was diluted with culture medium immediately before use. 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Human oral floor cancer cell line KB was obtained from American Tissue Culture Collection.

Cell culture

Cells were cultured in RPMI1640 (Invitrogen) containing 10% FBS (Hyclone), penicillin (50 U/ml), and streptomycin (50 U/ml) at 37 °C in a humidified atmosphere of 5% CO_2 incubator.

Immunofluorescence staining

Cells were seeded on cover slips, and incubated with VX-680 at 1 and 2 nM or DMSO (0.1%) for 24 h. The cells were washed in cold PBS and fixed in 2% *para*-formaldehyde-PBS at room temperature (RT) for 20 min and permeabilized in 0.5% Triton X-100 in PBS for 10 min at 4 °C. The cells were incubated with 1% BSA for 30 min at RT to block nonspecific binding before the primary antibody reaction. Slides were rinsed with PBS for three times. Then the cells were incubated with the primary antibody to Ser10-phosphorylated histone H3 (Cell signaling, #9706S), Aur-A (Upstate, #07-648), α-Tubulin (Sigma, T9026) at RT for 1 h, followed by a FITC or Alexa Flour 680 conjugated antibody, and then counterstained with DAPI (1 μg/μl), visualized using a microscope (Olympus BX51).

MTT assay

MTT assay was used to assess the growth of KB cells. The cells were plated in 96-well flat bottom plates at 1×10^4 cells/well in a final volume of 200 μl. When attached to the flat, the cells were exposed to different doses of VX-680 for 24 h. Sets of 4-wells were used for each dose and

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