Targeting Aurora kinase A suppresses the growth of human oral squamous cell carcinoma cells in vitro and in vivo

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Introduction

More than five hundred thousand new cases of head and neck squamous cell carcinoma (HNSCC) occurred in 2008 worldwide. Oral squamous cell carcinoma (OSCC) is the most frequently occurring cancer among HNSCC, with over a quarter of a million new cases of OSCC and a mortality rate of more than 50% reported in 2008. Despite improvement in our knowledge of the disease, as well as advances in chemotherapy, radiotherapy, and surgery, little improvement in the relative survival has been observed in OSCC during the past 40 years. Therefore, a greater understanding of the pathogenesis of OSCC is needed for the development of optimal therapeutic approaches.

Cancer cells acquire abnormalities in multiple oncogenes and tumor suppressor genes. Overexpression and constitutive activation of some oncogenes support the proliferation, invasion, and metastasis of cancer cells. Inactivation of a single critical oncogene can induce cancer cells to differentiate into cells with a normal phenotype or to undergo apoptosis. This dependence on oncogenes for maintaining the cancer phenotype provides an Achilles heel for tumors that can be exploited in cancer therapy. Recent experiences in humans indicate that it is possible to use pharmacological agents that inactivate oncogenes to treat at least some types of human cancer. For example, imatinib targeting BCR-ABL and KIT is used for patients with chronic myelogenous leukemia or advanced gastrointestinal stromal tumor. Thus, oncogene addiction has provided therapeutic opportunities in many human malignancies.

Materials and methods: Microarray analysis was performed to determine the gene expression profiles in nine human OSCC cell lines and a non-neoplastic keratinocyte cell line. The expression levels of Aurora kinase A (AURKA) mRNA and protein in human OSCC cells and tissues were examined. We investigated the effect of small interfering RNAs specific for AURKA (siAURKAs) and MLN8237, an AURKA selective inhibitor on the growth of OSCC cells in vitro and in vivo. We also analyzed clinical significance in AURKA mRNA expression levels in OSCC.

Results: AURKA was overexpressed in human OSCC cell lines and tissues. All siAURKAs almost completely suppressed the expression of AURKA protein, and significantly inhibited the growth of OSCC cells by 31–89%. MLN8237 also reduced the cellular growth rate by 38–74%. Both siAURKA and MLN8237 significantly reduced the size of subcutaneously xenografted OSCC tumors by 66% and 40%. Knockdown of AURKA expression and MLN8237 induced the growth inhibition of primary cultured cells established from patients’ OSCC tumors. Furthermore, we found a significant association between AURKA mRNA expression levels and histological differentiation and lymph node metastasis.

Conclusions: AURKA plays a critical role in the growth of human OSCC cells and targeting AURKA may be a useful therapeutic strategy for OSCC.

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such a molecule is a plausible therapeutic approach for the treatment of patients with OSCC. In this study, we focused on Aurora kinase A (AURKA), which had been reported the overexpression and amplification in various tumors including HNSCC. Furthermore, several AURKA selective inhibitors have been developed in the preclinical and clinical studies against solid tumors.

AURKA belongs to a family of serine/threonine kinase and plays an important role in centrosome function and duplication. In G2 to M phase, AURKA is involved in various mitotic events, centrosome maturation, centrosome separation, and mitotic entry. MLN8237, an AURKA selective inhibitor, is a second-generation oral inhibitor with an increased potency for inhibiting AURKA compared with MLN8054. MLN8237 binds to AURKA and inhibits the phosphorylation of Aurora kinase A, which results in the suppression of cell growth. Clinical phase studies of MLN8237 are proceeding against a wide range of solid tumors.

**Materials and methods**

**Cells and cell culture**

In this study, we used nine human OSCC cell lines, green fluorescent protein (GFP)-SAS, Ca9-22, HSC2, HSC3, HSC4, SCC111, SCC66, SCC9, and SCC25, and a human immortalized non-neoplastic keratinocyte cell line, HaCaT as described previously. These cells were maintained in DMEM supplemented with 10% FBS (Biosource, Camarillo, CA), 100 U/ml penicillin, and 100 µg/ml streptomycin (Wako), referred to here as complete medium. Primary cultured cells were established from patients’ OSCC tumors. Tumor tissues were surgically excised and prepared with 10 mM collagenase (Wako). The cell suspension was filtered through nylon mesh. The samples from patients with OSCC. In this study, we focused on Aurora kinase A (AURKA), which had been reported the overexpression and amplification in various tumors including HNSCC. Furthermore, several AURKA selective inhibitors have been developed in the preclinical and clinical studies against solid tumors.

AURKA belongs to a family of serine/threonine kinase and plays an important role in centrosome function and duplication. In G2 to M phase, AURKA is involved in various mitotic events, centrosome maturation, centrosome separation, and mitotic entry. MLN8237, an AURKA selective inhibitor, is a second-generation oral inhibitor with an increased potency for inhibiting AURKA compared with MLN8054. MLN8237 binds to AURKA and inhibits the phosphorylation of Aurora kinase A, which results in the suppression of cell growth. Clinical phase studies of MLN8237 are proceeding against a wide range of solid tumors.

**Small molecule compound**

MLN8237, an AURKA selective inhibitor, was purchased from Selleck Chemicals LLC (Houston, TX). For the in vitro use, it was dissolved in DMSO (Sigma–Aldrich, St. Louis, MO) to a stock concentration of 10 mM and stored at –80 °C until use. For the in vivo use, it was dissolved in 10% 2-hydroxypropyl-β-cyclodextrin (Sigma–Aldrich)/1% sodium bicarbonate (Wako) to a stock concentration of 10 mM.

**Samples from patients**

Fifty OSCC and four normal oral mucosa epithelial tissue samples from patients were obtained at the Ehime University Hospital from July 2006 to June 2012. OSCC tissues were collected from resected specimens of primary tumors (male, n = 28; female, n = 22; average age, 71.0) and normal oral mucosa epithelial tissues were derived from non-cancerous patients (male, n = 1; female, n = 3; average age, 32.8). Three primary cultured cells were derived from OSCC of the lower gingiva (male, 55 years, T4N2bM0), tongue (male, 57 years, T1N0M0), and lymph node metastasis (female, 74 years, T2N2bM0). The grade of tumor differentiation was determined according to the criteria proposed by the WHO. The Institutional Review Board (IRB) at Ehime University Hospital approved this study.

**Microarray analysis**

We used the Applied Biosystems Chemiluminescent RT-IVT Labeling Kit (Life Technologies) to convert total RNA to digoxigenin (DIG)-labeled cRNA. Total RNA was extracted by lysing the cells with the use of ISOGEN (NipponGene, Toyama, Japan). We used 1 µg of total RNA to generate the double-strand cDNA. The cDNA was transcribed with DIG-labeled nucleotides (Roche Diagnostics, Basel, Switzerland), fragmented, and hybridized to a Human Genome Survey Array (Life Technologies) according to the manufacturer’s instructions. After washing each array, we developed the signal by using a chemiluminescent detection kit (Life Technologies). Processed arrays were scanned with a 1700 Chemiluminescent Microarray Analyzer (Life Technologies).

We analyzed these results using the Genespring GX 12.1 (Agilent Technologies, Santa Clara, CA) and Ingenuity Pathway Analysis (IPA; Ingenuity Systems, www.ingenuity.com, Redwood City, CA) software. Functional analysis by IPA identified the biological functions or diseases that were most significant to the data set. Fischer’s exact test was used to calculate a p-value determining the probability that each biological function or disease assigned to that data set was due to chance alone. The microarray data are deposited in Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo, experiment number: GSE36090) according to minimum information about microarray experiment (MIAME) guidelines.

**Real-time quantitative reverse transcriptional polymerase chain reaction (qRT-PCR)**

Total RNA was extracted by lysing the cells or tissues with the use of ISOGEN (NipponGene). Tissues were homogenized in 1.0 ml of ISOGEN with the use of TissueLyser (Qiagen, Valencia, CA). The relative quantitation of the mRNA level using the comparative Ct method (ΔΔCt method) was carried out via qRT-PCR using the SYBR® system. Ribosomal protein, large, P0 (RPLPO) and hydroxymethylbilane synthase (HMBS) was used as an internal control. PCR amplification was performed in a 10-µl final reaction mixture containing 5 µl of 2 × One Step SYBR® RT-PCR Buffer 4, 0.4 µl of PrimeScript® One Step Enzyme Mix 2 (Takara, Otsu, Japan), 0.4 µl of forward primer (10 µM), 0.4 µl of reverse primer (10 µM), and 100 ng of total RNA. The thermal-cycling conditions were reverse transcribed at 42 °C for 5 min and 95 °C 10 s, followed by 40 cycles at 95 °C for 5 min and 60 °C 10 s. SYBR® Green I was detected with LightCycler (Roche Diagnostics). The sequences of primers used were as follows: AURKA: forward 5′-GAA ATT GCA AGC AAC CA-3′ and reverse 5′-GAG GGC GAC CAA TTT CAA AG-3′, RPLPO: forward 5′-CAA CCC TGA AGT CTA CAT-3′ and reverse 5′-AGG CAG ATG CAG CCC CAA-3′, HMBS: forward 5′-CAT GCA GCC TAC CAT CCA TGT C-3′ and reverse 5′-GGT AGC AGT GAT GCC TAC CCA-3′.

**Western blot analysis**

Cells (2.5 × 10⁵ for GFP-SAS, HSC2, and HSC4, 3 × 10⁵ for HSC3 and Ca9-22) transfected with siRNAs were grown in monolayers for 48 h and lysed with lysis buffer [0.5 M EDTA and 1% Triton-X (Sigma–Aldrich) in PBS containing a protease inhibitor cocktail and phosphatase inhibitor (Roche Diagnostics)]. Tissues were homogenized in 500 µl of lysis buffer with the use of TissueLyser (Qiagen). The samples were centrifuged at 15,000 g for 15 min at 4 °C, and supernatants were electrophoresed on SDS–polyacrylamide gels and transferred to polyvinylidene difluoride membranes (Millipore, Bedford, MA). The membranes were blocked with 5% non-fat dried milk (Wako) in 1 × TBS-T [25 mM Tris-HCl, 125 mM NaCl and 0.1% Tween 20 (Sigma–Aldrich)] for 1 h at...
AURKA protein expression levels were determined by Western blotting. The sequences of synthetic siGFP used were as follows: siAURKA-1: sense 5'-GAA GAG AAA GCA AAG CAA TT-3' and antisense 5'-UUG CUU UGC UUU UCU CTT-3', siAURKA-2: sense 5'-GAA CUU UCG UGG AUC ATT-3' and antisense 5'-UGA UCC AAG UAA GUU CTT-3', siAURKA-3: sense 5'-GAG UAA AGG UGA GCU ATT-3' and antisense 5'-UUG CAC AGC AGC UCC UUA ACU GTT-3', siNT: sense 5'-UAC GUA CUA UCG CGC -3 and antisense 5'-AUC GCC CGG ATA GUA CGU ATT-3'.

Cell growth assay

Cells (2 × 10^5 for GFP-SAS, HSC2, and HSC4, 3 × 10^5 for HSC3 and Ca9-22) were seeded into a 96-well plate in complete medium with 10 nM of synthetic siRNAs and 0.2% Lipofectamine RNAiMAX in a final volume of 100 μl. MLN8237 was added to each well to give a range of concentration (5–200 nM). After 72 h, the cell growth was evaluated by WST-8 assay (Cell counting Kit-8; Dojin-do, Kumamoto, Japan).

Xenograft model and tumor therapy

GFP-SAS cells (2 × 10^6) complexed with matrigel (BD) in 100-μl aliquots were injected subcutaneously at two sites in the flanks of male athymic nude mice (CLEA Japan, Tokyo, Japan). Two weeks later, tumor-bearing nude mice were randomly divided into treatment groups as follows: no treatment, siGFP, siAURKA-1, vehicle, or MLN8237. The final concentration of siRNAs was 40 μM in matrigel (AteloGene; Koken, Tokyo, Japan). These complexes were injected into tail veins every 3 days. MLN8237 (20 mg/kg) was received orally for 14 consecutive days. Tumor diameters were measured at regular intervals with digital calipers, and tumor volume (mm³) was calculated using the following formula: length × width × height × 0.523. Three mice were used in each group. Fifteen days after the first administration of siRNAs and MLN8237, GFP-SAS xenografts were dissected, and AURKA and p-AURKA protein expression levels were determined by Western blotting. The animal studies were approved by the Ehime University animal care committee. The sequences of synthetic siGFP used were as follows: sense 5'-CUA CAA CAG CCA CAA CGU CTT-3' and antisense 5'-GAC GUU GUG GUU GUA GCT-3'.

Statistical analysis

All in vitro experiments were performed in triplicate and repeated three times. Student's t-test was used to determine the significance of differences between the groups. P < 0.05 was considered statistically significant.

Results

Identification of oncogenic genes in human OSCC cells by microarray analysis

We determined the gene expression profiles in nine human OSCC cell lines and a non-neoplastic keratinocyte cell line. The total number of genes commonly up-regulated by more than 3-fold in nine human OSCC cell lines was 2345. Among these genes, 465 cancer-related genes were significantly identified by IPA (Supplementary Table 1). Subsequently, we selected 17 genes which had approval or investigational target drugs for cancer treatment (Fig. 1A, Table 1). Here, we focused on AURKA commonly overexpressed in human malignancies. The expression levels of AURKA in all human OSCC cell lines were more than 3-fold compared to that in the non-neoplastic keratinocyte cell line, HaCaT (Fig. 1B).

Expression of AURKA in human OSCC cells and tissues

We examined the expression of AURKA mRNA and protein in 5 human OSCC cell lines. The expression levels of AURKA mRNA and protein were higher in all human OSCC cell lines than in HaCaT and human normal oral mucosa epithelial primary cultured cells (Fig. 1C and D). Expression of AURKA mRNA was detected by qRT-PCR, whereas its protein expression was undetectable in HaCaT and human normal oral mucosa epithelial primary cultured cells by Western blotting. We compared the expression levels of AURKA protein in normal oral mucosa and OSCC tissues from the same patient and found higher expression of AURKA protein in the tumor tissues than in the normal tissues (Fig. 1E). These results suggested that AURKA mRNA and protein were overexpressed in human OSCC in not only cultured cells but also tissues. P-AURKA was not detected clearly in the OSCC tissues from patients (data not shown).

RNA interference (RNAi) and the growth inhibitory effects by siAURKAs in human OSCC cells in vitro

To clarify the function of AURKA in the cell proliferation of OSCC cells, we transfected synthetic siAURKA-1, -2, and -3 into GFP-SAS, Ca9-22, HSC2, HSC3, and HSC4 cells at the concentration of 10 nM to avoid off-target effects and interferon responses. All siAURKAs almost completely suppressed the expression of AURKA protein (Fig. 2A). Subsequently, we tested the effect of siAURKA-1, -2, and -3 on the growth of human OSCC cells. The knockdown of AURKA expression significantly inhibited the growth of these cells by 31–88% compared with siNT (Fig. 2B).

Effect of MLN8237 on the growth of human OSCC cells in vitro

We examined the effect of an AURKA selective inhibitor, MLN8237, on the growth of human OSCC cells. MLN8237 markedly reduced the growth rate of human OSCC cells. The growth of Ca9-22 and HSC2 cells was suppressed by 56–68% at the concentration of 50 nM MLN8237, but that of GFP-SAS, HSC3, and HSC4 cells was less than 50% at the concentration of 100 nM MLN8237 (Fig. 3A). The growth inhibitory effect of MLN8237 was slight compared to that of siAURKAs. To confirm the effect of MLN8237, we examined...
Figure 1 Identification of oncogenic genes in human OSCC cells. (A) Experimental scheme of microarray analysis. Using Human Genome Survey Arrays, we determined the gene expression profiles in nine human OSCC cell lines and 1 immortalized human non-neoplastic keratinocyte cell line, HaCaT. In all human OSCC cell lines, the expression of 2345 genes commonly up-regulated by more than 3-fold was detected. Among them, 465 genes were identified as cancer-related genes by IPA. AURKA was included in these genes. (B) Microarray relative signal intensity of AURKA in a non-neoplastic keratinocyte cell line, HaCaT, and nine human OSCC cell lines. The expression levels of AURKA mRNA in all OSCC cells were more than 3-fold compared to that of HaCaT. (C) Expression of AURKA mRNA in OSCC cell lines was evaluated by qRT-PCR. AURKA mRNA was overexpressed in human OSCC cells compared to HaCaT and human normal oral mucosa epithelial primary cultured cells. (D) AURKA protein expression in human OSCC cells was detected by Western blotting. In contrast, with little or no AURKA protein expression in HaCaT and human normal oral mucosa epithelial primary cultured cells, AURKA protein was detectable in all human OSCC cell lines. (E) AURKA protein expression in the tumor and normal tissues from the same patient was analyzed by Western blotting. Expression of AURKA in the tumor tissues was stronger than that of normal tissues. Bars denote standard deviation (SD) of samples performed in triplicate. S/N, signal/noise ratio; N, normal tissues; T, tumor tissues.

Table 1 Genes targeted by available anti-cancer drugs.

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<tr>
<th>Symbol</th>
<th>Gene name</th>
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<th>Target drug</th>
<th>FDA approval status</th>
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<td>HSP90AA1</td>
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FDA, Food and Drug Administration.
Figure 2 Knockdown and growth inhibitory effects of siAURKAs in human OSCC cells. (A) Three siAURKAs (10 nM) were transfected into human OSCC cell lines GFP-SAS, Ca9-22, HSC2, HSC3, and HSC4 with Lipofectamine RNAiMAX. The RNAi effect on AURKA protein was evaluated by Western blotting. All siAURKAs almost completely suppressed the expression of AURKA protein in all cells. (B) Human OSCC cell lines were seeded in complete medium with synthetic siRNAs. All siAURKAs significantly inhibited the growth of OSCC cells. *p < 0.01 compared to control culture. siNT, non-targeting siRNA.

Figure 3 Growth inhibition by MLN8237, an AURKA selective inhibitor, in human OSCC cells. (A) OSCC cell lines (GFP-SAS, Ca9-22, HSC2, HSC3, and HSC4) were treated with different concentration of MLN8237 (0, 5, 10, 20, 50, and 100 nM) for 72 h. Cell viability was evaluated by WST-8 assay. (B) GFP-SAS cells were treated with MLN8237 and transfected with siAURKA-1 for 24 h at the concentration of 100 nM and 10 nM, respectively. Western blot analysis was used to detect p-AURKA (Thr288), AURKA, and β-tubulin. MLN8237 inhibited the expression of p-AURKA and induced the accumulation of total AURKA protein. Bars denote the SD of samples performed in triplicate.
Figure 4 In vivo growth inhibitory effects of siAURKA and MLN8237. GFP-SAS cells (2 × 10⁶/50 µl) in equal volumes with matrigel were injected subcutaneously into the flank of nude mice. (A) Synthetic siAURKA/atelocollagen complexes were intravenously administrated into tail veins every 3 days and shown to significantly reduce the growth of GFP-SAS tumors in vivo (n = 6/group). (B) The expression levels of AURKA excised tumors were examined by Western blotting. Treatment with siAURKA reduced the expression of AURKA proteins in the tumor tissues. The number is the relative expression of AURKA in GFP-SAS tumors by densitometric analysis. (C) Nude mice bearing GFP-SAS tumors were dosed with MLN8237 orally at 20 mg/kg once daily for 14 days. MLN8237 significantly inhibited the GFP-SAS tumor growth (n = 6/group). *p < 0.01 compared to control group.

Figure 5 Effect of targeting AURKA in human OSCC primary cultured cells. (A–C) Primary cultured cells established from patients’ OSCC tumors were transfected with 10 nM of siAURKAs in Lipofectamine RNAiMAX. After 3 days, cell growth was evaluated by WST-8 assay. Cells transfected with siAURKAs were cultured for 48 h and lysed. The RNAi effect of siAURKAs was analyzed by Western blotting. Primary cultured cells were treated with different concentration of MLN8237 (0, 5, 10, 20, 50, 100, and 200 nM) for 3 days. Cell viability was evaluated by WST-8 assay. Knockdown of AURKA expression and MLN8237 inhibited the growth of OSCC primary cultured cells. Bars denote the SD of samples performed in triplicate. *p < 0.01 compared to control culture. siNT, non-targeting siRNA.
the expression of p-AURKA at threonine 288 by Western blotting. MLN8237 (100 nM) inhibited the phosphorylation of AURKA and consequently increased the total AURKA protein expression. Transfection of siAURKA (10 nM) almost completely suppressed the expression of both p-AURKA and total AURKA protein (Fig. 3B).

**Effect of siAURKA and MLN8237 on the in vivo growth of human OSCC cells**

We assessed the growth inhibitory effect of siAURKA and MLN8237 in vivo using a mouse model. We selected GFP-SAS cells for the in vivo assay because only these cells had tumorigenicity among the OSCC cells we used. We administered siAURKA/atelocollagen complexes into mouse tail veins every 3 days for a total of five injections. We found that these complexes significantly reduced the size of subcutaneously xenografted GFP-SAS tumors, compared with the control groups (Fig. 4A). Furthermore, the expression of AURKA in excised tumor tissues was notably suppressed by 66% in siAURKA/atelocollagen complex administration groups (Fig. 4B). When MLN8237 was administered orally at 20 mg/kg on 14 consecutive days to mice bearing GFP-SAS tumors, it also suppressed the size of tumors by approximately 40% (Fig. 4C). During administration of siRNA and MLN8237, no reduction of food intake or body weight was observed. Compared with the growth inhibitory effect of siAURKA, that of MLN8237 was slight. These in vivo data were similar to the data of growth inhibition of GFP-SAS cells in vitro.

**Effect of targeting AURKA in human OSCC primary cultured cells**

To confirm the usefulness of targeting AURKA in OSCC, we cultured the resected tumor tissues from three patients with OSCC and obtained the primary cultured cells. Primary cultured cells were derived from a lower gingiva tumor, a tongue tumor, and a lymph node metastasis, respectively. Subsequently, the in vitro growth inhibitory effects of siAURKAs and MLN8237 in primary cultured OSCC cells were examined. Three siAURKAs were transfected into primary cultured cells at the concentration of 10 nM. As in the case of OSCC cell lines, knockdown of AURKA expression induced the growth inhibition of OSCC primary cultured cells by 42–91% compared with siNT (Fig. 5A–C). MLN8237 also led to a dose-dependent decrease in the OSCC primary cultured cell growth (Fig. 5A–C).

**Clinical significance in the expression of AURKA mRNA**

Expression of AURKA mRNA in OSCC tissues resected from patients was examined. In 37 of 50 primary OSCC tissues (72.5%), the expression levels of AURKA mRNA in OSCC were more than 2-fold increase compared to normal oral mucosa tissues (Fig. 6A).

**Figure 6** Clinical significance in AURKA mRNA expression levels in OSCC. (A) Expression of AURKA mRNA in 50 primary OSCC tissues from patients was confirmed by qRT-PCR. Human oral normal mucosa tissues were used as a control. The relative quantitation of mRNA levels was calculated using the comparative CT method (2^\(-\Delta\Delta C_{T}\) method). (B) The relationship between AURKA mRNA expression and lymph node metastasis and differentiation in primary OSCC tissues was analyzed. The distinction according to lymph node metastasis, N (+), versus no metastasis, N (-), significantly affected AURKA mRNA expression (p = 0.0232). Compared to the differentiation of primary tumors, a significant difference was observed between poorly and well differentiated type (p = 0.0413). No significant association was observed between moderately and well, or between moderately and poorly differentiated type. NS, no significance.
Furthermore, we found a significant association between AURKA mRNA expression levels and histological differentiation and lymph node metastasis (Fig. 6B). The patients with high AURKA mRNA expression levels tended to show a poor prognosis, but the difference was not significant (data not shown).

Discussion

In microarray and IPA, we identified 17 cancer-related genes as candidates as potential molecular therapeutic targets for OSCC (Table 1). Some molecular targets, for example, ribonucleotide reductionase M2 (RRM2) targeted by gemcitabine, epidermal growth factor receptor (EGFR) targeted by cetuximab, and ABL1 targeted by imatinib, were included in these genes. In this study, we focused on AURKA but functional analysis of targeting other genes is ongoing. AURKA has been shown to be related to the progression, survival, histological differentiation, and metastasis in various tumors. In head and neck cancer, there is significant association between AURKA overexpression and progression or survival.\textsuperscript{13–15}

In addition, previous studies have reported that HNSCC cells and tissues overexpressed AURKA and knockdown of AURKA by siRNAs alone or combined with paclitaxel significantly reduced the growth of HNSCC cells in vitro.\textsuperscript{16} We also showed the overexpression of AURKA in OSCC as well as a clinically significant correlation between AURKA expression and histological differentiation and lymph node metastasis. Furthermore, we demonstrated the growth inhibitory effect of targeting AURKA by the use of siAURKA and MLN8237 on the growth of human OSCC cells in vitro and in vivo.

Overexpression of AURKA induces p53-dependent apoptosis in a mammary gland mouse model. P53 plays a critical role in the inhibition of tumor progression in the AURKA-overexpressed mammary gland. Loss of p53 is required for AURKA to induce tumorigenesis.\textsuperscript{17} Additionally, the retinoblastoma (Rb)/p16 pathway is involved in AURKA-induced senescence in a p53-deficient background. Neoplastic transformation by AURKA may require the disruption of both the p53/p21 and p16/Rb pathways.\textsuperscript{18} Recent whole-exome sequencing demonstrated that the mutations or deletions of p53 or p16 genes were frequently detected in HNSCC including OSCC.\textsuperscript{19,20} Therefore, we thought that targeting AURKA might be an appropriate therapeutic approach for OSCC patients.

A recent review showed that more than thirty small molecule inhibitors of Aurora kinase are undergoing preclinical and clinical studies.\textsuperscript{21} Among them, previous studies have shown that MLN8237 inhibits proliferation and leads to apoptosis in several human cancer cells.\textsuperscript{22–25} In animal models, MLN8237 has shown anti-tumor activity. The growth of nude mice xenograft colon cancer cells was remarkably inhibited by MLN8237 at 3, 10, and 30 mg/kg once daily for 21 consecutive days.\textsuperscript{26} Furthermore, phase I studies of MLN8237 in advanced solid tumors have been reported. The recommended phase II dose for MLN8237 is 50 mg twice daily for 7 days followed by 14-day recovery period, in 21-day cycles.\textsuperscript{26,27} In one of phase I studies, 3 cases of head and neck cancer were included, but to date there has been no report of anti-tumor activity by the use of MLN8237 in OSCC cells or tumors. Our study showed that treatment with MLN8237 significantly reduced the growth of human OSCC cells in vitro and in vivo. These results raise the possibility of MLN8237 as a novel therapeutic strategy for OSCC patients. The present study also demonstrated successful transfection of siRNA complexed with atelocollagen into xenografted tumor cells. Atelocollagen-mediated siRNA delivery has been reported to be effective in gene silencing following either local injection directly into tumors or intravenous systemic injection. This is because atelocollagen complexed with siRNA is resistant to nuclease, and it showed that siRNA can efficiently reach the target site in vivo, without being degraded by nuclease, if combined with an appropriate concentration of atelocollagen.\textsuperscript{28,29} Furthermore, our recent studies indicated that atelocollagen-mediated systemic administration of siRNA specific for androgen receptor and Akt1 resulted in the efficient inhibition of human prostate cancer cell growth without severe side effects such as lung, liver, or renal damage in nude mice.\textsuperscript{30,31} Administration of siAURKA also inhibited the growth of GFP-SAS tumors more than did MLN8237. These findings indicate that nucleic acid drugs such as siRNA may provide novel therapeutic opportunities in human cancer treatment.

In conclusion, AURKA functions as a critical gene for supporting the growth of human OSCC cells, and targeting AURKA appears to be a potentially useful therapeutic approach for patients with OSCC.

Conflict of interest statement

None declared.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.oraloncology.2013.02.002.

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