Clinicopathologic significance of EpCAM expression in squamous cell carcinoma of the tongue and its possibility as a potential target for tongue cancer gene therapy

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Summary Epithelial adhesion molecule (EpCAM) is a transmembrane glycoprotein involved in intercellular adhesion. In particular, EpCAM appears to be overexpressed by the majority of human epithelial carcinomas, including colorectal, breast, head and neck, and hepatic carcinomas. We therefore hypothesized that EpCAM would be a good molecular target for cancer gene therapy. EpCAM protein expression in 48 primary tongue cancers and 10 normal oral mucosa was evaluated using anti-EpCAM immunohistochemistry, and correlation was examined with the clinicopathologic factors. In four human tongue cancer cell lines (SAS, HSC-2, OSC19 and OSC20), we investigated EpCAM expression by reverse transcription-polymerase chain reaction (RT-PCR). The invasive potential of cancer cells was evaluated using Matrigel invasion assay. Moreover, the effect of EpCAM inhibition was analyzed using RNA interference (RNAi). EpCAM overexpression was detected in 30 of 48 tongue cancers (62.5%), and was significantly higher in primary squamous cell carcinoma (SCC) of the tongue than in normal oral mucosa. The expression of EpCAM was significantly associated with tumor size, regional lymph node metastasis, histological differentiation and invasion pattern. Cancer cell lines with higher EpCAM expression had more invasive potential. Moreover, RNAi-mediated EpCAM reduction decreased the invasion potential and proliferation activity. These results indicated that the overexpression

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Introduction

The primary modality for tongue cancer therapy is surgery. Although recent advancements in surgical techniques and anticancer agents have improved tumor regression and the survival rate, wide surgical resection of the tongue causes various oral dysfunctions; therefore, new treatment strategies are urgently needed.

The presence of neck regional lymph node metastasis is strongly related to a poor prognosis in squamous cell carcinoma (SCC) of the tongue. Some biomarkers predicting the poor prognosis of tongue cancer patients have been reported with several studies showing alterations of adhesion-related molecules in oral cancer. The epithelial cell adhesion molecule (EpCAM) is a 39–42 kDa, transmembrane glycoprotein that consists of two epithelial growth factor-like extracellular domains, a cysteine-poor region, a transmembrane domain, and a short cytoplasmic tail. EpCAM is detected at the basolateral membrane of the majority of epithelial tissues. EpCAM overexpression has been reported in cancers including colorectal cancer and breast cancer. EpCAM acts in Ca²⁺-independent intercellular adhesion, and is not structurally related to the four major types of cell adhesion molecules, such as cadherins, integrins, selectins, and the immunoglobulin superfamily. EpCAM overexpression in cancer cells induces the intracellular accumulation of cadherin–catenin complex, and cadherin-derived adhesion activity of cancer cells is consequently lost. Since the intercellular adhesive activity of EpCAM is very weak, the intercellular adhesion of cancer cells decreases, which may result in the acquisition of invasiveness and metastatic ability. In human breast cancer cells, a recent study suggested that the inhibition of EpCAM expression by short interfering RNA (siRNA) may decrease the availability of β-catenin for the Wnt pathway. However, the correlation of EpCAM overexpression and invasiveness or metastatic ability in cancer cells and its underlying mechanisms still remain unclear.

From the clinical point of view, the EpCAM antigen has attracted major interest as a target for cancer immunotherapy. Practically, the use of monoclonal antibody against EpCAM has been successfully used in colorectal cancer and breast cancer.

In this study, we investigated the usefulness of EpCAM overexpression as a clinical molecular marker, and the possibility of EpCAM targeting therapy for SCC of the tongue.

Materials and methods

Patients

Paraffin-embedded sections were obtained from biopsy specimens of 48 patients with squamous cell carcinoma of the tongue who underwent radical surgery in our department. The tumor stage was classified according to the TNM classification of the International Union Against Cancer. Tumor histologic differentiation was defined according to the WHO classification. The pattern of invasion was classified according to Byrnes’ classification. As controls, samples of normal oral epithelium were obtained after informed consent from ten patients undergoing routine surgical removal of their third molars.

Cell lines

The human tongue squamous cell carcinoma cell lines, SAS, HSC-2, OSC19 and OSC20 were obtained from the Human Science Research Resource Bank (Osaka, Japan). All of the cells were cultured under conditions recommended by their depositors.

Immunohistochemical staining and evaluation

Serial sections 3 μm thick were taken from the tissue blocks. Deparaffinized sections in xylene were soaked in target retrieval solution buffer (DAKO, Glostrup, Denmark) and placed in an autoclave at 121 °C for 5 min for antigen retrieval. Endogenous peroxidase was blocked by incubation with 0.3% H₂O₂ in methanol for 30 min. Immunohistochemical staining was performed using the Envision system (EnVision+, DAKO, Carpinteria, CA). The primary antibody used was directed against EpCAM (HEA-125, GeneTex, San Antonio, TX; 1 μg/ml concentration). The sections were incubated with the monoclonal antibody overnight at 4 °C. Reaction products were visualized by immersing the sections in diaminobenzidine (DAB) solution, and the samples were counterstained with Meyer’s hematoxylin and mounted. Negative controls were performed by replacing the primary antibody with phosphate-buffered saline. EpCAM expression was defined as the presence of specific staining on the surface membranes of tumor cells. EpCAM overexpression was evaluated by calculating the total immunostaining score as the product of the proportion score and the intensity score. The proportion score described the estimated fraction of positive-stained tumor cells (0, none; 1, <10%; 2, 10–50%; 3, 50–80%; 4, >80%). The intensity score represented the estimated staining intensity (0, no staining; 1, weak; 2, moderate; 3, strong). The total score ranged from 0 to 12. As described previously, overexpression of EpCAM was defined as a total score >4.

RNA isolation and semiquantitative reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was isolated with TRizol Reagent (Invitrogen, Carlsbad, CA) and first-strand cDNA was synthesized from
EpCAM expression in tongue cancer

1 μg total RNA using Oligo d (T) primer (Invitrogen) and ReveTrna Ace (TOYOBO, Osaka, Japan). For PCR analysis, cDNA was amplified by Taq DNA polymerase (TAKARA, Otsu, Japan). Glycerinaldehyde-3-phosphate dehydrogenase (GAPDH) was used as the endogenous expression standard. Each PCR program involved a 3-min initial denaturation step at 94°C, followed by 28 cycles (for EP-CAM and β-catenin), or 18 cycles (for GAPDH) at 94°C for 30 s, 55°C for 30 s and 72°C for 1 min, on a PCR Thermal Cycler MP (TAKARA). Primer sequences were for, EP-CAM: F, CAGAACAAATGATGGGCTTTT and R, AGCGTTGTTGATCTCCTTC; for β-catenin: F, TGGCCTTGTGTTGATCCAGCCT and R, CTCACAGCCCATCACATTG; and for GAPDH: F, ATGTCAGGTGGTACTGCTGC and R, TGACCTGGCCCAACGCTTTG. The amplified products were separated by electrophoresis on ethidium bromide-stained 2% agarose gels. Band intensity was measured by NIH Image 1.63.

Invasion assay

The BioCoat Matrigel invasion chamber (Becton Dickinson, Bedford, MA) contains an internal chamber with an 8-μm porous membrane bottom and is coated with Matrigel. Six-well cell culture inserts and a six-well multiwell companion plate were used for this experiment. The membranes were rehydrated with warm serum-free medium for 2 h. The internal chamber was filled with 1.25 × 10⁵ cells in medium containing 5% FBS. The lower chamber was filled with medium containing 10% FBS as a chemoattractant. Cells were incubated for 72 h at 37°C in a 5% CO₂ atmosphere. After incubation, noninvading cells were removed from the top of the wells with a cotton swab, and cells that transferred to the invasive surface of the membrane were subjected to Diff-Quick staining. The cells were counted under a microscope at 100× magnification. For the control cell count, cells that passed through a control chamber without Matrigel were counted. All experiments were completed in triplicate, and at least 4 fields/well were counted. The percentage of the cell count that passed through the Matrigel chamber to the control count was calculated as the invasion index.

RNA interference (RNAi)

All siRNAs were purchased from Greiner Japan (Tokyo, Japan). Cells were transfected with double-strand RNA using Oligofectamine reagent (Invitrogen) according to the manufacturer's protocol. The OSC19 tongue cancer cell line was used for this experiment. Briefly, 2.5 × 10⁴ OSC19 cells were plated in each well of six-well plates and allowed to grow for 24 h (until they were approximately 50% confluent). Short interfering RNA targeting was then transfected into cells at a concentration of 200 nM using Oligofectamine reagent and serum-free medium. After 4 h of incubation, serum-rich medium was added. For the design of siRNA oligos targeting EpCAM, we selected siRNA sequences as reported by Osta et al. The EpCAM siRNA sequences were 5'-GUUACCGCCACCGCCGAUdTdT-3' and 5'-CUACACGUGCCUGUAAACdTdT-3'. The scrambled control siRNA sequences were 5'-ACUGACACCGUUCGGA-GAAdTdT-3' and 5'-UUUCGCCAGGUCGACGUdTdT-3'. All sequences were submitted to the National Institutes of Health Blast program to ensure gene specificity.

Western blot analysis

For the extraction of membrane proteins from cells, the Mem-Per Eukaryotic Membrane Protein Extraction Reagent Kit (Pierce Chemical Co., Rockford, IL) was used. Cells were harvested by trypsinization, washed and pelleted by centrifugation. The cell pellets were lysed at room temperature using cell lysis buffer. The membrane proteins were solubilized on ice with membrane solubilization buffer diluted 1:1 with detergent dilution buffer. The solubilized protein mixture was centrifuged to remove cellular debris. The clarified supernatant was heated at 37°C for 10 min following centrifugation to produce separate membrane and hydrophilic protein fractions. Phase partitioning resulted in the hydrophilic proteins layering at the top and the hydrophobic membrane proteins layering at the bottom. The cytoplasmic and nuclear proteins were purified using NE-PER Nuclear and Cytoplasmic Extraction Reagents (Pierce Chemical Co.). All subsequent manipulations were performed on ice. The cells were incubated in the cytoplasmic extraction reagent. The lysed cells were centrifuged for 5 min to pellet the intact nuclei, and the supernatant was removed (cytoplasmic fraction). The pelleted nuclei were resuspended in the nuclear extraction reagent, vortexed vigorously for 15 s and incubated on ice for 40 min with periodic vortexing every 10 min. At this point, the mixture was centrifuged for 10 min, and the supernatant was removed (nuclear fraction). The protein concentration of each sample was measured with micro-BCA protein assay reagent (Pierce Chemical Co.). The samples were denatured in SDS sample buffer and loaded onto 12.5% polyacrylamide gels. After electrophoresis, the proteins were transferred onto a polyvinylidene difluoride membrane and immunoblotted with anti-EpCAM (HEA-125, GeneTex, San Antonio, TX; 0.02 μg/ml concentration) or anti-β-catenin (9F2, Abcam, Cambridge, UK; 0.02 μg/ml concentration). The signal was detected using the horseradish peroxidase-conjugated secondary antibody (ECL antimouse IgG, Amersham Biosciences, Piscataway, NJ; 0.01 μg/ml concentration), and then visualized using an ECL Kit (Amersham Pharmacia Biotech, Buckinghamshire, UK).

Cell proliferation assay

Cells were seeded in the 96-well plate at a concentration of 5 × 10³ per well and incubated for 24 h. The cells were transfected with EpCAM siRNA or the scrambled control siRNA, followed by incubation for 0, 24, 48, 72, and 96 h. At each time point, cells were incubated with 0.5 mg/ml 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma Chemical Co., St Louis, MO). Four hours later, the medium was replaced with 100 μl dimethylsulfoxide (DMSO) (Sigma Chemical Co.) and vortexed for 10 min. Absorbance was then recorded at 570 nm using Easy Reader 340 AT (SLT-Lab Instruments, Salzburg, Austria).
Statistical analysis

Statistical analyses were performed using StatMate III (Atms Co., Tokyo, Japan). The associations between EpCAM expression and clinicopathologic features were assessed by Fischer’s exact test. To determine significant prognostic factors related to survival, multivariate analysis was performed using the Cox proportional hazards regression model. The continuous data were given as the mean ± standard deviation. Data sets were examined by one-way analysis of variance (ANOVA) followed by Scheffe’s post-hoc test. The correlation between EpCAM mRNA expression and the invasion index was estimated by Pearson’s correlation coefficient. P values less than 0.05 were considered significant.

Results

Correlation of EpCAM overexpression and clinicopathologic features

Immunohistochemistry with an anti-EpCAM-specific monoclonal antibody was evaluated in a series of 48 patients with SCC of the tongue. Representative immunohistochemical stainings are shown in Figure 1. Although the overexpression of EpCAM was undetectable, focal EpCAM positivity was shown in normal epithelium (Fig. 1A). A strong EpCAM staining with high intensity was shown in the invasive front of SCC cells (Fig. 1D). EpCAM overexpression was detected in 30 of 48 SCCs of the tongue (62.5%). EpCAM overexpression was significantly more frequent in SCC of the tongue than in normal oral epithelium (P = 0.0007, Table 1). Moreover, EpCAM overexpression was correlated with tumor stage (P = 0.033), regional lymph node metastasis (P = 0.005), histologic differentiation (P = 0.027) and pattern of invasion (P = 0.034, Table 1).

Cox regression analysis was performed with the parameters of T classification, N classification, histologic differentiation, pattern of invasion, and EpCAM overexpression. There were no significant independent predictors of survival (Table 2); however, patients without EpCAM overexpression showed a weak trend toward better survival. Eighty-three percent of patients without EpCAM overexpression, but 73% of patients with EpCAM overexpression, lived longer than 5 years. These data were analyzed using the Kaplan–Meier method and compared using the log-rank test (data not shown).

EpCAM is associated with invasion potential of tongue cancer cells

EpCAM mRNA expression of tongue cancer cell lines, SAS, HSC-2, OSC19, and OSC20 were analyzed by semiquantitative RT-PCR. The expression of EpCAM mRNA was detectable in all tongue cancer cell lines, and the EpCAM/GAPDH intensity ratio varied from 0.21 to 0.85 (Fig. 2A). To characterize the invasion potential of tongue cancer cells in vitro, we used Matrigel invasion chambers. The highest invasion index was noted in OSC19, followed by SAS and OSC20 with similar
Table 1: Correlation of EpCAM overexpression and clinico-pathologic features

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Number of samples</th>
<th>EpCAM overexpression</th>
<th>P value</th>
</tr>
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<tbody>
<tr>
<td>Normal epithelium</td>
<td>10</td>
<td>0</td>
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<tr>
<td>SCC</td>
<td>48</td>
<td>18</td>
<td>0.033</td>
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<tr>
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<td>0.201</td>
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<tr>
<td>Female</td>
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<td>0.178</td>
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<td>&gt;60</td>
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<td>T1 + T2</td>
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<tr>
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<td>2</td>
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<tr>
<td>N classification</td>
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<tr>
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<tr>
<td>Well</td>
<td>41</td>
<td>18</td>
<td>0.034</td>
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<tr>
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<td>0</td>
<td></td>
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<tr>
<td>Pattern of invasion</td>
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<tr>
<td>Grades 1/2</td>
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<td>14</td>
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</tr>
<tr>
<td>Grades 3/4</td>
<td>20</td>
<td>4</td>
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</table>

SCC, squamous cell carcinoma.

indices, and the index was lowest in HSC-2 (Fig. 2B). The EpCAM mRNA expression level was significantly correlated with the invasion index of the tongue cancer cells (r = 0.808, P = 0.001, Fig. 2C).

Down-regulation of EpCAM blocks nuclear translocation of β-catenin

To determine the possibility of EpCAM targeting therapy for tongue cancer, the tongue cancer cell line OSC19 with high EpCAM expression and high invasiveness was treated with EpCAM siRNA as well as with scrambled siRNA. The control was treated with Oligofectamine reagent alone. Scrambled siRNA transfection had no effect on EpCAM mRNA expression. Moreover, β-catenin total mRNA expression was unaffected by transfection with either EpCAM or scrambled siRNA, and obvious reduction in EpCAM mRNA was observed with EpCAM siRNA at a final concentration of 200 nM (Fig. 3A).

Table 2: Multivariate analysis (Cox regression) of different prognostic parameters

<table>
<thead>
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<th>Parameter</th>
<th>Hazard ratio</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
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<tr>
<td>T classification (T1 + T2 versus T3 + T4)</td>
<td>1.52</td>
<td>0.77–3.02</td>
<td>0.23</td>
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<tr>
<td>N classification (N0 versus N1 + N2)</td>
<td>1.46</td>
<td>0.67–3.14</td>
<td>0.34</td>
</tr>
<tr>
<td>Differentiation (Well versus Moderate/Poor)</td>
<td>1.25</td>
<td>0.52–2.03</td>
<td>0.62</td>
</tr>
<tr>
<td>Pattern of invasion (Grades 1/2 versus Grades 3/4)</td>
<td>1.03</td>
<td>0.53–2.00</td>
<td>0.93</td>
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<tr>
<td>EpCAM overexpression (− versus +)</td>
<td>0.81</td>
<td>0.39–1.70</td>
<td>0.58</td>
</tr>
</tbody>
</table>

95% CI, 95% confidence interval.
Furthermore, to examine the effect of EpCAM siRNA treatment on the localization of β-catenin protein, the fractionated membrane, cytoplasmic and nuclear proteins were analyzed by immunoblotting. In our study, the membrane and cytoplasmic fractions of β-catenin clearly increased in response to EpCAM siRNA treatment. In contrast, the nuclear fraction of β-catenin decreased in response to EpCAM siRNA treatment (Fig. 3B).

**Down-regulation of EpCAM significantly decreases the invasion potential of OSC19 cells in vitro**

To determine the effect of EpCAM siRNA treatment on the invasion potential, we transfected OSC19 cells with EpCAM siRNA, and analyzed using Matrigel invasion assay. Transfection with EpCAM siRNA significantly decreased the invasive cells in OSC19 (P < 0.01, Fig. 3C). The mean invasion index of cells transfected with EpCAM siRNA was 11.1%, whereas the means of the invasion index of cells treated with Oligofectamine reagent alone and transfected with scrambled siRNA were 55.2% and 52.3%, respectively.

**Down-regulation of EpCAM significantly decreases the proliferation of OSC19 cells**

The effect of EpCAM siRNA treatment on the proliferation of OSC19 was analyzed using MTT assay. The control was treated with Oligofectamine reagent alone. The scrambled siRNA was used as a control for nonspecific effects of siRNA treatment on cell growth. The number of viable cells was determined at 0, 24, 48, 72, and 96 h using MTT assay. Transfection with EpCAM siRNA significantly decreased the proliferation in OSC19 at 24, 48, 72, and 96 h (Fig. 3D).

**Discussion**

This study examined the overexpression of EpCAM in biopsy specimens of human tongue cancer patients using immunohistochemistry. EpCAM is currently known to be overexpressed in various cancers. Several reports demonstrated that EpCAM expression would be useful as a molecular marker of the critical tumor border or carcinogenesis. In this study, we observed EpCAM overexpression in OSC19 cells. The present study showed that EpCAM siRNA treatment significantly decreased cell proliferation and invasion in OSC19 cells transfected with EpCAM siRNA (P < 0.01). EpCAM siRNA treatment significantly decreases cell proliferation. The OSC19 cells were plated on 96-well plates. After 24 h incubation, the cells were transfected with EpCAM siRNA or the scrambled control siRNA, followed by incubation for 0, 24, 48, 72, and 96 h. Cell viability was determined using MTT assay. The data are presented as the means of three separated experiments, each performed in triplicate; bars, SD. ○, control cells that were not treated with siRNA; ▽, cells treated with scrambled siRNA; ◇, cells treated with EpCAM siRNA.
pression in 30 of 48 SCCs (62.5%), whereas it was not observed in any normal epithelia. Therefore, we suggest that EpCAM overexpression is associated with tongue carcinoma.

In colon, gastric, prostate and lung cancer, no obvious correlation of EpCAM overexpression with survival, tumor stage, nodal stage or grade was observed. However, some studies reported that the overexpression of EpCAM was an independent marker of poor survival in breast, gall bladder and esophageal cancers. On the other hand, patients with EpCAM overexpressing clear cell renal carcinoma showed a trend toward a better prognosis. Hence, the clinicopathologic significance of EpCAM overexpression is controversial. Some investigators reported that EpCAM expression in breast cancer was correlated with several clinicopathologic parameters, such as high tumor grade, large tumor size or the presence of axillary lymph node metastases. In squamous cell carcinoma of the lung, EpCAM expression increases with worsening grade, TNM stage and prognosis. In this study, we demonstrated that EpCAM overexpression in tongue cancer could reflect a large tumor size, regional lymph node metastasis, less differentiated tumor, and diffuse invasion. These results strongly support the conclusion that EpCAM affects the progression of tongue cancers; however, in our study, EpCAM expression was not a prognostic factor in tongue cancer patients.

In this study, we demonstrated that EpCAM expression could be associated with invasiveness in human tongue cancer cell lines. Our study indicated that the de novo overexpression of EpCAM increased the invasion potential of tongue cancer cells. Moreover, the immunohistochemical staining of EpCAM revealed strong positivity in the invasive front of the diffuse invasion pattern; however, the mechanism by which EpCAM increases invasive potential is still unclear. Some studies have demonstrated that EpCAM overexpression decreases adhesion mediated by the cadherin—catenin complex. Catenin links cadherin with actin cytoskeleton, and can also form a complex with epidermal growth factor receptor. β-catenin, a multifunctional protein, is involved in major functions, such as cell adhesion and mediation of the Wnt signal pathway, which consists of highly conserved secreted ligands that bind cell-surface receptors called frizzled and lipoprotein receptor-related protein. In the presence of Wnt signaling, β-catenin is accumulated in the cytoplasm, translocated into the nucleus, and interacts with the DNA-binding T-cell factor complex and acts as a transcriptional activator, thus turning on target genes, including c-myc, vascular endothelial growth factor, cyclooxygenase 2, and others, which are involved in development and oncogenesis. Thus, we focused on the intracellular localization of β-catenin, measuring β-catenin protein in the fractionated membrane, cytoplasmic and nuclear proteins, respectively. To inhibit specific EpCAM, we used chemically synthesized siRNA to achieve direct homology-dependent posttranscriptional gene silencing. Our results revealed that EpCAM siRNA significantly inhibited the invasiveness of OSC19 cells. Furthermore, the translocation of β-catenin into the nucleus of OSC19 cells was blocked by EpCAM siRNA transfection. In addition to this blocking nuclear translocation of β-catenin, we have shown that the transfection of EpCAM siRNA induces the redistribution of β-catenin to the cell membrane, and increases the cytoplasmic accumulation of β-catenin. In contrast with our results, Osta et al. reported that EpCAM gene silencing using RNAi, as well as our technique, induced a significant decrease in cytoplasmic β-catenin. Although this discrepancy is unknown, cytoplasmic β-catenin may be degraded as a recent study reported that only β-catenin complexed with membrane E-cadherin is protected from degradation. Therefore, we speculate that the results of Osta et al. caused the rapid degradation of cytoplasmic β-catenin by deficient binding to membrane E-cadherin. Consequently, we support the idea that EpCAM might promote cellular invasion via the Wnt/β-catenin pathway.

To evaluate the effect of EpCAM siRNA on cellular proliferation, we examined OSC19 cells using MTT assay and found that EpCAM down-regulation significantly inhibited the proliferation of OSC19 cells. Some authors have reported that EpCAM can be associated with cellular proliferation. 12,34 and a recent study reported that EpCAM upregulates c-myc, cyclins and epithelial fatty acid binding protein, which induces cellular proliferation. These transcriptional factors, including c-myc and cyclin D1, are activated by the nuclear translocation of β-catenin. As mentioned above, we indicated that EpCAM siRNA could block the nuclear translocation of β-catenin, and we therefore suggest that blocking the nuclear translocation of β-catenin by EpCAM siRNA treatment inactivates transcriptional factors, such as c-myc and cyclin D1, and inhibits the cellular proliferation of OSC19 cells.

Recently, EpCAM was highlighted as a target antigen for cancer immunotherapy. In fact, several monoclonal antibodies such as edrecolomab and ING-1 were used in advanced cases of colorectal cancer and pancreatic adenocarcinoma; however, the antitumor efficacy of these monoclonal antibodies is limited. Edrecolomab in combination with 5-FU based chemotherapy revealed a small improvement in overall survival compared with 5-FU based chemotherapy alone in the adjuvant treatment of stage III colon cancer. It has recently been reported that RNAi technology is a specific and powerful tool to turn off the expression of oncogenic target genes. In oral cancer, the possibility of RNAi-mediated gene therapy has been reported. 44,45 We successfully applied RNA silencing to inhibit the expression of EpCAM, and its effect decreased the invasion potential and cellular proliferation of tongue cancer cells. Therefore, we suggest that RNAi-mediated gene silencing of EpCAM can be a useful modality for tongue cancer treatment.

Conflict of interest

We have no potential conflicts of interest.

Acknowledgement

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