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Abstract: The newly identified MMP-28 has been shown to be expressed in several types of carcinomas, however, none of its functional role in transformation events is known. This study was to assess
whether this proteinase plays a role in oral tumor malignancy. By using RT-PCR, we found that incidence of MMP-28 was significantly higher in 92 oral squamous cell carcinomas (OSCCs) (52/92, 56.5%) than in 7 oral premalignant lesions (OPMLs) (0/7, 0%) (P = 0.004). No statistically significant correlation was found

between MMP-28 expression and tumor stage, thickness, size, and metastasis. Both mRNA and protein of MMP-28 were preferentially concentrated in OSCC specimens than in neighboring tissues as analyzed by semi-quantitative RT-PCR (P = 0.015) and immunohistochemistry, respectively. Transfection of an OSCC and an esophageal carcinoma cell lines with MMP-28 antisense oligodeoxynucleotide (AODN) resulted in the reduced secretion of MMP-28 protein and the ability of colony formation in soft agar without affecting cell
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growth. Our findings show the close correlation between MMP-28 and OSCC, and support a role for MMP-28 in the anchorage-independent growth of both OSCC and esophageal carcinomas.
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Cover letter

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Revision Notes

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members. This “ Product Description" is shortly described in the Result (p10), and detailed in the Materials and Methods (p6) in the revised manuscript.
3. Western blotting of the conditioned medium of the esophageal carcinoma cell line, CE81T/VGH, is included in Fig. 3A.
4. The recombinant MMP-28 protein is not available within 2 months.
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2. Lohi J, Wilson CL, Roby JD, Parks WC. Epilysin, a novel human matrix metalloproteinase (MMP-28) expressed in testis and keratinocytes and in response to injury. J Biol Chem. 2001 Mar 30;276(13):10134-44.
3. Questions of MMP-28 antibody to and answers from Sigma:
Q: I've submitted a manuscript to "Oral Oncology" last week. Their reviewers suggested this is not an acceptable paper unless I can show the specificity of the antibody used in this study (anti-MMP-28, M 5066). Did you use recombinant MMP-28 and other recombinant MMP proteins to test the specificity of M 5066? It will be very much appreciated if you can send me any immunoblotting data of M 5066 for its QC ASAP.
Ans: Thank you for contacting Sigma-Aldrich Technical Service. The information we have on product M5066, Anti-Matrix Metalloproteinase-28, indicates the following: The peptide-purified antibody does not recognize the other MMP family members (MMP-1, MMP-2, MMP-3, MMP-9, etc.).
The mouse sequence is predicted to yield a 70.077 kDa protein. Promoter analysis suggests that MMP-28 may be constitutively produced in some cells, or that regulation is different from most other MMPs. MMP-28 also has a furin cleavage site, like MMP-11, and is likely cleaved by the prohormone convertase family of enzymes. Sequence homology between MMP-28 and other MMPs is low overall, most closely resembling MMP-19
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(at 39% identity). RP4MMP-28 Anti-MMP-28 works well for Western Blotting applications, and when used against the reduced protein identifies bands at 62 Kd and 58 Kd, as well as breakdown products at 50, 48 and 46 kD. A larger band at 64 kD and a smaller band at 30 kD are also seen in some cell lines. Please feel free to contact me if you have any further questions.
Q:Thanks for your rapid response. Could you tell me how did your experts know: "the peptide purified antibody does not recognize the other MMP

family members (MMP-1, MMP-2, MMP-3, MMP-9, etc.)"? What kind of experiments have they done?
A: I have had a response from my colleagues on product M5066 (anti-MMP-28, C-terminal, developed in rabbit), regarding the specificity. This follows up to the previous messages:
a. The MMP-28 antibodies were made to synthetic peptides to the different domain epitopes of MMP-28.
b. The MMP-28 sequence was aligned with the other 24 MMP sequences. The modeling of the peptides was such that there was very little overlap in sequence homology between MMP-28 and the other MMPs.
c. The MMP-28 antibodies were tested by Western blot using lysates and culture media from cell lines and tissues that we use to test the other MMP antibodies. There was no significant cross reactivity against the other MMPs.
d. We did not have a source of purified MMP-28, either recombinant or native, at the time, but we did use keratinocytes, and they gave a positive signal at the appropriate size."
Hopefully this will help you in the discussion with your paper referee.

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Manuscript

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2Abstract
The newly identified MMP-28 has been shown to be expressed in several types of carcinomas, however, none of its functional role in transformation events is known. This study was to assess whether this proteinase plays a role in oral tumor malignancy. By using RT-PCR, we found that incidence of MMP-28 was significantly higher in 92 oral squamous cell carcinomas (OSCCs) (52/92, 56.5%) than in 7 oral premalignant lesions (OPMLs) (0/7, 0%) (P = 0.004). No statistically significant correlation was found between MMP-28 expression and tumor stage, thickness, size, and metastasis. Both mRNA and protein of MMP-28 were preferentially concentrated in OSCC specimens than in neighboring tissues as analyzed by semi-quantitative RT-PCR (P = 0.015) and immunohistochemistry, respectively. Transfection of an OSCC and an esophageal carcinoma cell lines with MMP-28 antisense oligodeoxynucleotide (AODN) resulted in the reduced secretion of MMP-28 protein and the ability of colony formation in soft agar without affecting cell growth. Our findings show the close correlation between MMP-28 and OSCC, and support a role for MMP-28 in the anchorage-independent growth of both OSCC and esophageal carcinomas.
Keywords: Matrix metalloproteinase, MMP-28, Oral squamous cell carcinoma, esophageal carcinoma, colony formation

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3Introduction
Matrix metalloproteinases (MMPs) play a central role in many physiological functions, such as development, wound healing, inflammation, and angiogenesis.1-4 Over the past two decades, the relevance of the MMP family in the oncology has been considerably studied. These enzymes were associated with the invasive properties of tumor cells, owing to their ability to degrade all major protein components of the extracellular matrix (ECM) and basement membranes. Elevated levels of some certain MMPs can be detected in tumor tissues or sera of patients with advanced cancers. They can also serve as prognostic indicators in various cancers, such as the head and neck carcinomas.7,8
Inhibition of tumor growth, migration, invasion, and metastasis by specific MMP inhibitors or antisense strategies has been demonstrated in numerous studies. For instances, expression of MMP-9 antisense construct inhibited the growth and invasion of a human glioblastoma cell line.9,10 A newly synthesized gelatinase inhibitor, ONO-4817, alone or combined with docetaxel, suppressed the metastatic potential of MMP-expressing lung carcinoma cell lines in immunodeficient mice.11,12 Moreover, a cyclic peptide CTTHWGFTLC, selected from a phage display peptide libraries, demonstrated to specifically inhibit the activities of MMP-2 and MMP-9, blocked the migration of human endothelial and tumorous cells as well as to prevent tumor growth and invasion in animal models.13
Up-to-date, MMP-28 (epilysin), structurally belonging to the MMP-19 subfamily, may represent the newest MMP member and is expressed in a variety of normal and carcinoma tissues.14,15 In wounded human skin, MMP-28 protein is prominently stained in basal keratinocytes both at and some distance from the wound edge. It was also present in fetal tissues and rhesus monkey placenta during early pregnancy.14,16

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4Therefore, its function in normal tissue homeostasis, wound repair, and development, as well as in tumor progression is suggested.14-16 MMP-28 may also be endowed with immunological functions, as it was found in T lymphocytes from the blood of normal individual, and elevated in the cartilage from patients with osteoarthritis.17,18 These findings further amplify the diversified functions of MMP-28.
The involvement of MMP-28 in pathological processes remains obscure. For examples, in the inflammatory conditions, it was shown to be upregulated in osteoarthritis by immunohistochemistry (IHC),17 but downregulated in inflammatory

bowel disease or ischemic colitis by real-time PCR.19 Furthermore, MMP-28 was demonstrated to be widely expressed in a variety of carcinomas, such as pancreatic adenocarcinoma, ovarian carcinoma, and colon adenocarcinoma.14 On the other hand, this proteinase was shown to be downregulated in colon cancers, and its function in tissue homeostasis rather than in tumor progression was emphasized.19
To assess whether MMP-28 plays a role in oral tumors, we firstly examined the expression pattern of MMP-28 transcript in surgical specimens of 7 oral premalignant lesions (OPMLs) and 92 oral squamous cell carcinomas (OSCCs) by RT-PCR. Distribution of MMP-28 protein was also analyzed by IHC in another 10 OSCC tissue sections. To explore its possible functions, an OSCC cell line, Meng-1 (OECM-1),20 and an esophageal carcinoma cell line, CE81T/VGH,21,22 were subjected to specific inhibition of MMP-28 by antisense oligodeoxynucleotide (AODN) transfection. Cell proliferation and anchorage-independent growth were subsequently analyzed.

,5Materials and methods
Tumor specimens
Surgical specimens of 7 oral premalignant lesions (OPMLs) and 92 oral squamous cell carcinomas (OSCCs) were collected from Chi-Mei Medical Center and preserved at -80°C for subsequent RNA preparation. Twenty-four of the 92 OSCC specimens as well as their neighboring tissues were simultaneously collected for the comparison of MMP-28 mRNA level between the malignant and the surrounding tissues.
Semi-quantitative RT-PCR
Protocols of tissue homogenate, total cellular RNA preparation and RT-PCR conditions were described in our previous work.23,24 Briefly, the primer sequences and respected sizes of PCR products of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and MMP-28 genes are listed in Table 1. Briefly, total cellular RNA (4 μg) was reverse-transcribed into the first strand cDNA, and one tenth of the reverse-transcribed cDNA was further subjected to PCR amplifications. Cycle numbers of PCR were 24 and 30 for GAPDH and MMP-28, respectively, to insure the generation of sub-maximal products. Amplified products were separated by electrophoresis with 100 bp DNA Ladder (GeneRulerTM, MBI Fermentas, Vilnius, Lithuania) in 1.5% agarose gels and then visualized under UV light after being stained with 0.5 μg/ml ethidium bromide.
Immunohistochemistry
Slides from 10 OSCC sections were deparaffinized in xylene, followed by absolute ethanol, 95% ethanol, and distilled water. Before immunostaining procedures, sections were incubated for 30 min in 10 mM sodium citrate buffer (pH 6.0) at 100°C to enhance the immunoreactivity of samples. A commercial anti-MMP-28 polyclonal

,6antibody (M5066) was used to detect MMP-28 immunoreactivity (Sigma Diagnostics, Inc., St. Louis, Missouri, USA), followed by biotinylated goat anti-rabbit antibody (Dako, Glostrup, Denmark), and revealed by the avidin-biotin complex (Vectastain kit, Vector, Burlingame, California, USA) and the 3-amino-9-ethylcarbazole (AEC) chromogen. Note that M5066 is a newly generated antibody against human MMP-28. In fact, there are 4 MMP-28 antibodies (M5066, M4566, M4691, and M5191) simultaneously developed by immunizing rabbits with peptides from 4 distinct regions of human MMP-28. All of these 4 antibodies recognize 58 kDa and 62 kDa molecules by immunoblotting against the reduced protein in several cell lines. That they does not cross react with other MMP family members, such as MMP-1, MMP-2, MMP-3, and MMP-9 is described by the manufacturer (Sigma Diagnostics, Inc.). Thus, M5066 is thought to interact with MMP-28 specifically.
Transfection of cells with oligodeoxynucleotides
According to the reported MMP-28 cDNA sequence, phosphorothioate-modified AODN synthesized covers from 2 codons upstream to 3 codons downstream from the start codon: 5'-GCCGACGCGCGCGACCATCTCGCC-3'. As a control, the sense oligodeoxynucleotide (SODN) was synthesized within the same region as: 5'-GGCGAGATGGTCGCGCGCGTCGGC-3'. Cells were seeded at a density of 1 × 106 cells/well in a 6-well plate in 2 ml Dulbecco's modified Eagle's medium (DMEM) (Gibco BRL, Paisley, UK) containing 10% fetal bovine serum (FBS) (HyClone, Logan, Utah, USA). After 24 h, culture medium was removed and the cells were washed twice with 2 ml PBS, and re-suspended in 1 ml serum-free DMEM (SFM) with or without AODN or SODN (0.2 μM) in the form of complex with Lipofectamine (GibcoBRL) as instructed by the manufacturer. The transfection protocols were repeated for subsequent detection of MMP-28 protein or measurement

,7of cellular proliferation, 2-D motility, and soft-agar-colony-formation assay.
Western blot analysis
After the transfection, cells were cultured in DMEM containing 30% FBS for 24 h. Afterwards, cells were washed twice with 2 ml PBS and were cultured in 2 ml SFM for 24-72 h. The 2 ml SFM was then collected as the conditioned medium (CM), and was then subjected to centrifugal concentration by passing through the 10,000 MWCO PES membrane (Vivascience, Hannover, Germany) as described by the manufacturer. The final concentrated volumes each CM were averagely 60 μl, and 15 μl was subjected to sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) with 10% acrylamide gel (Bio-Rad Laboratories, Inc., Hercules,

California, USA). Separated proteins were transferred onto nitrocellulose (NC) paper (Amersham Pharmacia Biotech, Uppsala, Sweden). The NC paper was immediately soaked in 10-fold diluted Ponceau S solution (Sigma Diagnostics, Inc.) for 1 min and photographed. The NC paper was then extensively washed with H2O and subsequently blocked with 5% lipid-extracted milk at room temperature for 1 h, and immunoblotted with 0.2 μg/ml of anti-MMP-28 (M5066) antibody in TBS buffer (50 mM Tris-HCl, pH 7.35, 0.85% NaCl) containing 0.5% lipid-extracted milk at room temperature for 1 h. After washing three times with TBS washing buffer [50 mM Tris-HCl, (pH 7.35), 0.85% NaCl, 0.5% Tween 20], the NC paper was further incubated with 1 x 104-fold diluted goat-anti-rabbit-IgG antibody coupled with horseradish peroxidase (Chemicon, International, Inc., Temecula, California, USA) in the same buffer as the first antibody at room temperature for 1 h. This immunoblotted paper was then extensively washed with TBS buffer, and signals of MMP-28 protein were detected with WESTERN LIGHTENINGTM Chemiluminescence Reagent (PerkinElmer Life Sciences, Inc., Boston, Massachusetts, USA) as instructed by the

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8manufacturer.

Proliferation assay

Cellular proliferation was determined by using a XTT labeling reagent, Cell Proliferation Kit II (Roche Molecular Biochemicals, Indianapolis, Illinois, USA), as instructed by the manufacturer. Briefly, 1 × 104 cells/well of SODN or AODN transfected OECM-1 and CE81T/VGH cells were seeded onto a 96-well plate, and incubated for 6 days. Afterwards, these cells were incubated with XTT labeling reagent for 3 h. Each experiment was performed in triplicate and repeated twice.

Soft-agar-colony-formation assay

SODN or AODN transfected OECM-1 or CE81T/VGH cells were plated onto each well of a 6-well plate at a density of 5 × 104/well in 2 ml DMEM containing 20% FBS and 0.2% agarose (with 0.5% agarose underlay). The number of colonies was determined at 6 days following plating.

Statistical analyses

Difference between the MMP-28 incidence in OPMLs and OSCCs was analyzed by Fisher ' s Exact Test. Two groups of data presented as mean ± SD were analyzed by Student ' s t test. A value of $P < 0.05$ was regarded as statistically significant.

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9Results

Incidence of MMP-28 in OPMLs and OSCCs

By using the RT-PCR analysis, MMP-28 message was preferentially detected in OSCCs (52/92, 56.5%) rather than in OPMLs (0/7, 0%) ($P = 0.004$, Table 2). Twenty-four of the 52 MMP-28-positive OSCC samples (T1~T24) are shown in Figure 1A. On the other hand, MMP-28 incidence was not statistically correlated with tumor stage, thickness, size, and lymph node metastasis (Table 2).

Elevated transcription of MMP-28 in OSCCs

To compare the expression level of MMP-28 between OSCCs and their surrounding tissues, T1~T24 and their neighboring tissues (N1~N24) were subjected to semi-quantitative RT-PCR analysis (Fig. 1A). PCR conditions were designed to generate sub-maximal products of MMP-28 and GAPDH genes, and the intensity of their signals were densitometrically determined. Each of the Nx and Tx ratio, representing the relative expression level of MMP-28, was calculated by (MMP-28 density of Nx/GAPDH density of Nx) and (MMP-28 density of Tx/GAPDH density of Tx), respectively (x = 1~24). The average ratio of T1~T24 (Tav) was calculated to be 2.9 ± 3.3, which is significantly greater than that of N1~N24 (Nav = 1.4 ± 2.2) ($P = 0.015$) (Fig. 1B). These results implied that MMP-28 is mainly transcribed within OSCC region.

Concentrated localization of MMP-28 protein in OSCCs

To further analyze whether the distribution of MMP-28 protein is also concentrated within OSCC region, tumor sections from another 10 OSCC patients were subjected to immunohistochemical staining of MMP-28. The results of these 10 sections were similar that MMP-28 protein was mainly stained within tumor region. One of the 10 results is shown in Fig. 2.

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Effects of MMP-28 AODN transfection

The expression pattern of MMP-28 suggested a close correlation with OSCC. Therefore, analysis of various phenotypic changes of tumor cells after the specific inhibition of MMP-28 protein expression by transfecting cells with MMP-28 AODN was utilized to further examine its possible roles in OSCC. To assess the transfection efficiency, the secreted MMP-28 protein level in the CM of OECM-1 and CE81T/VGH cells after MMP-28 SODN and AODN transfection was measured by Western blot. The results showed that the secretion of MMP-28 was 68% and 57% reduced in AODN transfected OECM-1 and CE81T/VGH, respectively, than in those of SODN transfected cells (Fig. 3A). The newly developed anti-MMP-28 antibody (M5066) used in this study identifies bands at 58 kDa and 62 kDa (thought to be the authentic protein products of MMP-28 gene) in several cell types, including keratinocytes, and does not cross react with other MMP family members (detailed in Materials and Methods). Therefore, the doublet signals at 58 kDa and 62 kDa as

detected from the CM of OECM-1 and CE81T/VGH (Fig. 3A) are regarded as the secreted MMP-28 protein.
To further address whether transfection of MMP-28 AODN interferes cell growth and colony formation in soft agar, both SODN and AODN transfectants of OECM-1 were subjected to XTT assay and soft-agar-colony-formation assay. The results showed that AODN transfection did not affect cellular proliferation of this cell line (Fig. 3B). In contrast, the colony forming ability of OECM-1 was significantly attenuated by the transfection of AODN than that of SODN (P = 0.046) (Fig. 3C, left).
To analyze whether the inhibition of MMP-28 AODN in anchorage-independent growth can be applied to other type of upper aerodigestive tract (UADT) carcinoma, another human esophageal carcinoma cell line, CE81T/VGH, was subjected to the

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same experiments as OECM-1. Similar results were obtained that the growth of CE81T/VGH was not affected (Fig. 3B), while the colony numbers formed in soft agar was significantly decreased in AODN than in SODN transfectants (P = 0.009) (Fig. 3C, right).

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Discussion
According to our present study, MMP-28 message was preferentially expressed in OSCCs (52/92, 56.5%) than in those of OPMLs (0/7, 0%) (P = 0.004, Table 2), and was dominantly transcribed in OSCC specimens than in those of neighboring tissues (p = 0.015, Fig. 1). In consistent with the distribution of mRNA, MMP-28 protein was also mainly concentrated in OSCCs than in those of surrounding tissues (Fig. 2). These expression patterns suggested that MMP-28 might play an important role in OSCC. It is speculated that, after progressed from OPML to OSCC, MMP-28 is required throughout the entire progression process in 50~60% OSCCs, which may explain why the incidence of MMP-28 was not statistically correlated to the tumor stage, size, thickness, and metastasis (Table 2). However, there may be still 40~50% OSCCs progressed in an MMP-28-independent manner.
Factors involved in cell growth, motility, colony formation, invasion, and metastasis may be separable. For examples, Hauck et al. demonstrated the differential dependence of cellular growth/motility and invasion/metastasis on focal adhesion kinase (FAK)/Src-mediated signals by stable expression of the FAK C-terminal domain (FRNK) in v-Src-transformed NIH 3T3 fibroblasts. The invasion through Matrigel and the metastases in nude mice, but not the growth and 2-D motility, were blocked in FRNK transfected cells.25 Moreover, Park et al. showed that low concentration level (0.1 μM) of Taxol almost abolished the ability of lymphoblastic leukemia cells to form colony in soft agar without evident effect on cell growth.26 Our antisense studies indicated that MMP-28 is required for the colony formation of both OECM-1 and CE81T/VGH cells, but is not directly involved in cellular proliferation (Fig. 3B) and 2-D migration (analyzed by scratching test, data not shown). This is the first demonstration by the in vitro evidence showing the participation of MMP-28 in

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the anchorage-independent growth of carcinoma cells. Inhibition of the colony forming ability of CE81T/VGH by MMP-28 AODN suggested that functions of this proteinase in tumorigenicity are not only restricted to OSCC, but also can be applied to other type of UADT carcinomas.
Processing of growth factors required for anchorage-independent growth by MMP-28 may be one of the possible underlying mechanism, since tumor necrosis factor-α and heparin-binding EGF-like growth factor are known to be processed by MMPs.27,28 There are also growing evidences indicating the correlation of small leucine-rich proteoglycans, such as lumican and decorin, with colony formation of transformed cells. Li et al. illustrated that cleavage of lumican by membrane-type 1 MMP (MT1-MMP) abrogated this proteoglycan-mediated suppression of tumor cell colony formation in soft agar.29 Suppression of decorin expression was also found to be related to the induction of anchorage-independent growth caused by v-src in human fetal lung fibroblasts.30 It is thus possible for MMP-28 to improve the colony forming capacity of tumor cells by processing such factor(s).
As mentioned earlier, the expression of MMP-28 may be differentially regulated in different tumor types.14,19 The diverse expression pattern was also observed in its subfamily member, MMP-19, which was firstly demonstrated to be downregulated in invasive area of squamous cell carcinomas in chronic wounds, neoplastic and dedifferentiated skin cancers by Impola et al.,31,32 and in invasive mammary gland tumors by Djonov et al..33 However, Impola et al. have further shown that MMP-19 was expressed by epithelial keratinocytes in hyperproliferative areas of verrucous hyperplasia, verrucous carcinoma, and OSCC in a more recent study.34 Thus, together with our present studies, it is concluded that the expression of this particular MMP subfamily (MMP-19 and MMP-28) is not inhibited in oral carcinomas. Recent studies

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on MMP-19 may have further confirmed this proteinase to be a cancer-related MMP.
For example, by using the transgenic mice model, Pendas et al. demonstrated that,

MMP-19-deficient mice exhibited decreased susceptibility to skin tumors induced by methylcholanthrene (MCA). These mice developed fewer fibrosarcoma and with a longer latency period than wild-type littermates after a typical MCA induction protocol.35 Together with our present studies, these results validated the possibility that members of MMP-19 subfamily may play a role in tumorigenesis.

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Figure Legends
Figure 1. Comparison of MMP-28 level between oral squamous cell carcinomas (OSCCs) and their neighboring tissues. Twenty-four surgical specimens of OSCCs (T1~T24) and their neighboring tissues (N1~N24) were subjected to semi-quantitative RT-PCR analysis of MMP-28 and GAPDH genes. RT-PCR results of N1~N24 and T1~T24 were shown in (A). Signals of these two genes were digitalized spectrophotometrically and the MMP-28/GAPDH ratio of each specimen was calculated. The average MMP-28/GAPDH ratios of N1 ~N24 (Nav) and T1~T24 (Tav) were presented as mean ±SD and plotted in (B).
Figure 2. Localization of MMP-28 in the tissue section of oral squamous cell

carcinoma (OSCC). This OSCC section was immunohistochemically stained with a pre-immuned rabbit serum (A-C) or with a polyclonal antibody against MMP-28 (D-F). Scale bars = 400 μm (A,D), 200 μm (B,E), and 100 μm (C,F).
Figure 3. Effects of the transfection of sense and antisense oligodeoxynucleotides (SODN and AODN) of MMP-28 on cell growth and colony formation. (A) Secreted MMP-28 protein in the conditioned media of OECM-1 and CE81T/VGH was measured by Western blot after MMP28 SODN and AODN transfection. (B) Cellular proliferation of OECM-1 and CE81T/VGH was assessed by XTT assay after MMP-28 SODN and AODN transfection. (C) Colony numbers of MMP-28 SODN or AODN transfected OECM-1 and CE81T/VGH cells were scored, and data are presented as mean ± SD.

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Figure 1A

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Figure !B

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Figure 3A

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Table

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Table 2 Incidence of MMP-28 in 7 oral premalignant lesions (OPMLs) and in distinct tumor characteristics of cell carcinomas (OSCCs)

Stage (n = 92)	Thickness (n = 90)	Size (n = 92)2	NLNM+(OPML: 0/7 (0%)1
MMP-28−: stage I: 6/12 (50%)	6-10 mm: 16/32 (50%)	T2: 15/31 (48%)	MMP-28+: stage II: 10/19 (53%)
11-15 mm: 9/13 (69%)	T3: 6/12 (50%)		
stage III: 6/12 (50%)	>15 mm: 16/29 (55%)	T4: 24/35 (69%)	
stage IV: 30/49 (61%)			

1Incidence of MMP-28 as analyzed by RT-PCR.
2T1~T4 were classified by the UICC/AJCC TNM classification system (2002).
3Thirty-five of 88 OSCC patients were examined to be neck-lymph-node metastasized (NLNM+). MMP-28+ or MMP-positive or negative results of RT-PCR, respectively.
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