Oral Diseases (2006) 12, 27–33. doi:10.1111/j.1601-0825.2005.01151.x

http://www.blackwellmunksgaard.com

ORIGINAL ARTICLE

Increase of MMP-13 expression in multi-stage oral carcinogenesis and epigallocatechin-3-gallate suppress MMP-13 expression

W-C Chiang[1], Y-K Wong[1,2], S-C Lin[1], K-W Chang[1], C-J Liu[1,3,4]

[1]*The School of Dentistry, National Yang-Ming University Taipei, Taiwan;* [2]*Department of Dentistry, Taichung Veterans General Hospital, Taiwan;* [3]*Department of Oral and Maxillofacial Surgery, Taipei Mackay Memorial Hospital, Taipei;* [4]*Mackay Junior College of Nursing, Taipei, Taiwan*

BACKGROUND: Matrix metalloproteinases (MMPs) play pivotal roles in tumor progression. MMP-13 (collagenase-3) digests collagen and other extracellular components.
MATERIALS AND METHODS: Reverse transcriptase-polymerase chain reaction (RT-PCR), immunohistochemistry and zymograph were used to study the roles of MMP-13 during the neoplastic process of oral squamous cell carcinoma (OSCC).
RESULTS: Increase of *MMP-13* mRNA and protein expression in OSCC cell lines relative to cultivated normal oral keratinocytes was found. *MMP-13* mRNA expression in OSCC was significantly higher than in non-cancerous match tissue (NCMT) in 36 tissue pairs. Esophageal squamous cell carcinoma also exhibited high *MMP-13* mRNA expression. The percentage of OSCC exhibiting strong MMP-13 immunoreactivity was significantly higher than pre-invasive lesion and NCMT. Treatment with >5 μM epigallocatechin-3-gallate (EGCG) to OEC-M1 cells suppressed the expression and activity of MMP-13.
CONCLUSION: MMP-13 could be a potential tumor marker for OSCC. The effects of EGCG in tumor inhibition may act partially through the modulation of MMP-13.
Oral Diseases (2006) **12**, 27–33

Keywords: MMP-13; mouth; neoplasm; oral carcinoma; precancerous lesion

Correspondence: Chung-Ji Liu, Department of Oral and Maxillofacial Surgery, Mackay Memorial Hospital, No 92, Sec. 2, Chungshan North Road, Taipei, Taiwan. Tel: 886-2-2543-3535 ext 2216, Fax: 886-2-2543-3642, E-mail: cjliu@ms2.mmh.org.tw
Received 13 December 2004; accepted 15 April 2005

Introduction

Degradation of basal lamina and stromal extracellular matrix (ECM) is crucial for invasion and metastasis of malignant cells. Studies reveal that matrix metalloproteinases (MMPs) play pivotal roles in tumor invasion (Curran and Murray, 1999). The human MMP comprises a family of homologous zinc-dependent endopeptidases that are capable of degrading ECM components (Khokha and Denhardt, 1989). This enzyme family participates in matrix remodeling processes associated with development, growth, wound healing, inflammation, and tumor invasion (McCawley and Matrisian, 2000; Chang and Werb, 2001).

Members of the collagenase subfamily of MMP were divided into MMP-1 (fibroblast collagenase, collagenase-1), MMP-8 (neutrophil collagenase, collagenase-2), and MMP-13 (collagenase-3) that degrades type I–III fibrillar collagens (Westermarck and Kahari, 1999). In addition, MMP-13 also degrades type IV collagens and a wide variety of ECM (Knauper *et al*, 1997). This diverse proteolytic capacity of MMP-13 suggests a role in ECM remodeling with tumor progression. Increased expression of MMPs has been found to be associated with local invasion or lymph node metastasis in oral squamous cell carcinoma (OSCC) or head and neck squamous cell carcinoma (HNSCC) (Johansson *et al*, 1997a; Kurahara *et al*, 1999; Thomas *et al*, 1999; Dunne *et al*, 2003). However, the roles of MMP-13 in oral tumorigenesis were not clearly addressed. We identified *MMP-13* as a tumor-enriching component in our previous subtractive hybridization library analysis (Lin *et al*, 2002). This study investigated the expression of MMP-13 in multi-stage oral carcinogenesis using cultivated cells and tissues.

The beneficial properties of green tea polyphenol in the inhibition of mutagenesis, tumor growth, invasion and metastasis have been documented (Taniguchi *et al*, 1992). Epigallocatechin-3-gallate (EGCG), the main

green tea polyphenol, is a potent inhibitor for the expression and activity of MMP-2 and -9 (Garbisa *et al*, 2001; Maeda-Yamamoto *et al*, 2003). The impacts of EGCG on MMP-13 at mRNA, protein and activity levels were also explored in this study.

Materials and methods

Cell culture

Primary normal human oral keratinocytes (NHOKs) were obtained from patients undergoing flap operation for removal of impacted wisdom teeth as approved by Institutional Review Board (IRB) and cultured in KSFM (Life Tech., Gaithersburg, MD, USA) (Lin *et al*, 2002). OEC-M1, OC-3 and SCC25 OSCC cell lines were cultured following standard protocols (Lin *et al*, 2002,2004).

Samples

Surgical specimens from 36 OSCCs and paired non-cancerous match tissue (NCMT) were obtained as approved by IRB. The age of the patients ranged from 29 to 76 years with a mean of 49 years. The most common primary site was buccal mucosa (50%, 18 cases). In histopathological grading, 14% (5/36) of OSCC showed good differentiation and 86% (31/36) showed moderate or poor differentiation. Fifty-three percent (19/36) of the patients presented with lymph-node metastasis. Fifty-eight percent (21/36) of patients had stage IV OSCC. Immunohistochemistry (IHC) analysis was performed on tissue sections of 44 OSCCs, 21 oral pre-invasive lesions (OPLs), and 16 NCMT. The OPL showed hyperplasia, hyperkeratosis or epithelial dysplasia. Thirty-eight percent (eight cases) of OPL exhibited mild or moderate epithelial dysplasia. Forty-two percent (six cases) of NCMT had hyperplasia or hyperkeratosis. No patients with NCMT showed epithelial dysplasia.

IHC

The IHC procedures followed previously established protocols (Chang *et al*, 2002). Anti-MMP-13 monoclonal antibody (Oncogene, Boston, MA, USA) with 1:200 dilution was used as primary antibody. For the negative controls, the primary antibody was omitted. A total of five random high-power (×400) fields were examined on each section for scoring immunoreactivity. Specimens containing $<10\%$ positive cells were defined as $-$ (negative) for immunoreactivity. Specimens containing 10–50%, and $\geq 50\%$ positive cells were defined as $+$ (weak) and $++$ (strong) for immunoreactivity, respectively.

EGCG treatment

Epigallocatechin-3-gallate (Sigma, St Louis, MI, USA) was dissolved in Dimethyl sulfoxide (DMSO) and added to cultures using the concentration for various time periods (Maeda-Yamamoto *et al*, 2003). The effect of EGCG on the vitality of OEC-M1 cells was determined by MTT assay (Ko *et al*, 2003). For reverse transcriptase-polymerase chain reaction (RT-PCR), Western blot and gelatin zymography analysis, the conditioned media were concentrated using Amicon centrifugal filter devices (Millipore, Bedford, MA, USA). The monolayer OEC-M1 cells were harvested, counted and subjected to RNA isolation.

RT-PCR

Total RNA isolation and reverse transcription reaction were conducted using the protocols previously used (Chang *et al*, 2002). Sense primer: 5′-CTATGGTC-CAGGAGATGAAG-3′ and antisense primer: 5′-AGA-GTCTTGCCTGTATCCTC-3′ were used to generate 390-bp MMP-13 amplicons. Amplification of a 190-bp glyceraldehyde 3-phosphate dehydrogenase (*G3PDH*) amplicon was used as an internal control. After electrophoresis, the densities of the amplicons were measured by a densitometer (Amersham, Piscataway, NJ, USA). The signal of *MMP-13* was normalized with *G3PDH* to represent the *MMP-13* mRNA expression.

Western blot

Conditioned media were collected and electroblotted onto nitrocellulose membranes following a standard protocol (Lin *et al*, 2002). The dilution for anti-MMP-13 antibody (Oncogene) and anti-G3PDH antibody (Chemicon, Temecula, CA, USA) was 1:200 and 1:5000, respectively. Horseradish peroxidase-conjugated anti-mouse antibodies (Amersham) were used as secondary antibody. Autoradiography signals were detected by a chemoluminescence detection system (Amersham) and analyzed with ImageQuant software (Amersham).

Gelatin zymography

Conditioned media were mixed with sodium dodecyl sulfate (SDS) sample buffer without heating or reduction to preserve enzyme activity. The samples were electrophoresed in 10% polyacrylamide gels copolymerized with 0.1% gelatin according to documented methods (Maeda-Yamamoto *et al*, 2003). After electrophoresis, the gels were washed for SDS removal, renaturing of the MMPs and incubated with $CaCl_2$ and $ZnCl_2$. The gels were subsequently stained with 0.1% coomassie blue, and cleared areas of gelatinolytic activity were shown on the blue background after destaining.

Statistics

Paired *t*-test and Fisher's exact test were used for statistical analysis. Results with $P < 0.05$ were considered to have statistically significant difference.

Results

MMP-13 mRNA expression

MMP-13 amplicon was detected in three distinctive OSCC cells, but it was barely detectable in two NHOKs (Figure 1a). No *MMP-13* amplicon was detectable in three additional NHOKs (data not shown). Proform (60 kDa) or active form (48 kDa) of *MMP-13* were present in cell lysate of OSCC cells, and they were barely

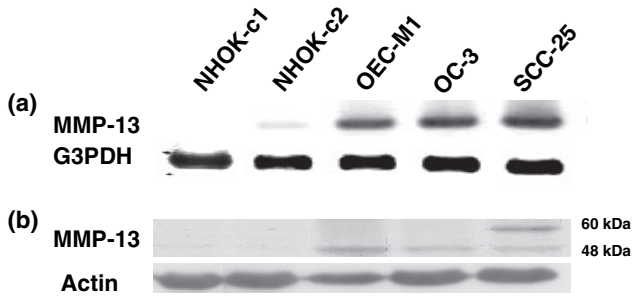

Figure 1 Analysis of oral keratinocytes. (**a**) RT-PCR. *MMP-13* mRNA was srongly expressed in three OSCC cell ines. It was weakly expressed in NHOK-c2 and completely absent in NHOK-c1. Amplicons of *G3PDH* used as controls. (**b**) Western blot. Both MMP-13 proform (60 kDa) and active form (48 kDa) were present in cell lysate of SCC-25, while only active MMP-13 was detected in OEC-M1 and OC-3 cells. No MMP-13 was detectable in any NHOK. Note that OEC-M1 had the most aboundant active MMP-13 among OSCC cell lines. Actin served as a control. Results shown are representative of two independent experiments

detectable in NHOKs (Figure 1b). Among 36 OSCC/NCMT pairs, *MMP-13* amplicon was detected in around 90% (32/36) of OSCC (Figure 2), but only 36% (13/36) of NCMT. In addition, nearly all OSCC exhibit amplicon signals much higher than the corresponding NCMT. Analysis of 10 esophageal squamous cell carcinoma (ESCC)/NCMT pairs also showed congruent results. The *MMP-13* mRNA expression was 1.83 ± 0.21 for OSCC and 0.45 ± 0.12 for NCMT. A significant difference in *MMP-13* mRNA expression lies between these two groups ($P < 0.0001$, paired *t*-test,) (Figure 3a). However, there was no correlation between *MMP-13* mRNA expression and clinicopathological features, including sites, clinical stage, differentiation grade, lymph node metastasis and patients' age (detailed analysis not shown). The *MMP-13* mRNA expression was 2.10 ± 0.41 for ESCC and 0.10 ± 0.02 for NCMT with a significant difference ($P < 0.001$, paired *t*-test) (Figure 3b).

MMP-13 immunoreactivity

Immunohistochemistry of MMP-13 was performed on sections of NCMT, OPL and OSCC. Cytoplasmic MMP-13 immunoreactivity was seen in epithelial cells, inflammatory cells and stromal cells in tissues (Figure 4). In addition, scattered nuclear MMP-13 immunoreactivity was also detected in epithelial cells. Of 16 NCMT, six (38%) displayed strong immunoreactivity (++), nine (56%) weak immunoreactivity (+) and only one (6%) showed no immunoreactivity (–). MMP-13 immunoreactivity was remarkably stronger in OSCC counterparts of these NCMT (Figure 4a–d). All (44 cases) OSCC revealed strong MMP-13 immunoreactivity (Figure 4b,d). Among 21 OPLs, five (24%) displayed strong immunoreactivity, eight (38%) weak immunoreactivity, and eight (38%) showed the absence of immunoreactivity (Figure 4e,f). Five of eight (63%) epithelial dysplasia exhibited strong MMP-13 immunoreactivity. The remaining three (37%) had weak MMP-13 immunoreactivity. Heterogenous nuclear MMP-13 immunoreactivity was seen in three (21%) NCMT, six (29%) OPL and 12 (27%) OSCC. Fourteen OSCC exhibited distinguishable increases in MMP-13 immunoreactivity in their invasion fronts (Figure 4g). The IHC profile of multi-stage oral carcinogenesis is summarized in Figure 5. OSCC was significantly different from NCMT and OPL in MMP-13 immunoreactivity ($P < 0.0001$, Fisher's exact test). However, the difference between NCMT and OPL was insignificant.

Effects of EGCG on MMP-13

As fetal calf serum (FCS) in culture medium may interfere with zymographic detection of MMP-13, the analysis of MMP-13 in supernatant was performed under FCS-free conditions. MTT assay showed that >40 μM EGCG treatment for 24 h caused the cytotoxicity of OEC-M1 cells (detailed analysis not shown). Thereby, 20 μM EGCG was defined as the maximal dosage for OEC-M1 treatment. RT-PCR was used to measure the *MMP-13* mRNA expression following

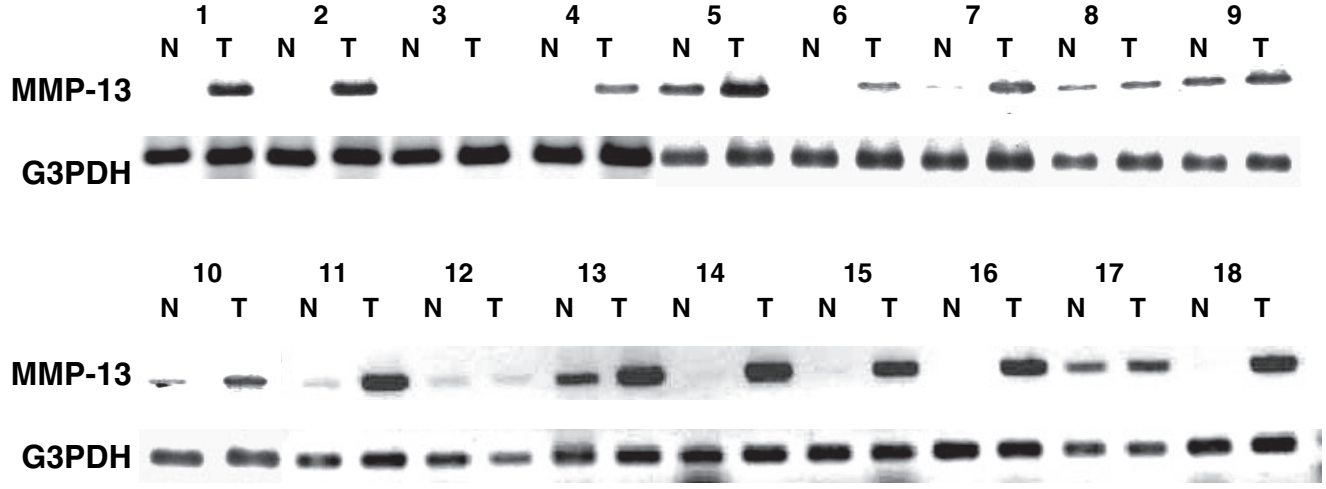

Figure 2 RT-PCR analysis of oral tissue pairs. *MMP-13* mRNA expression was generally higher in OSCC than in corresponding NCMT samples. *G3PDH* served as controls. N: NCMT; T: OSCC

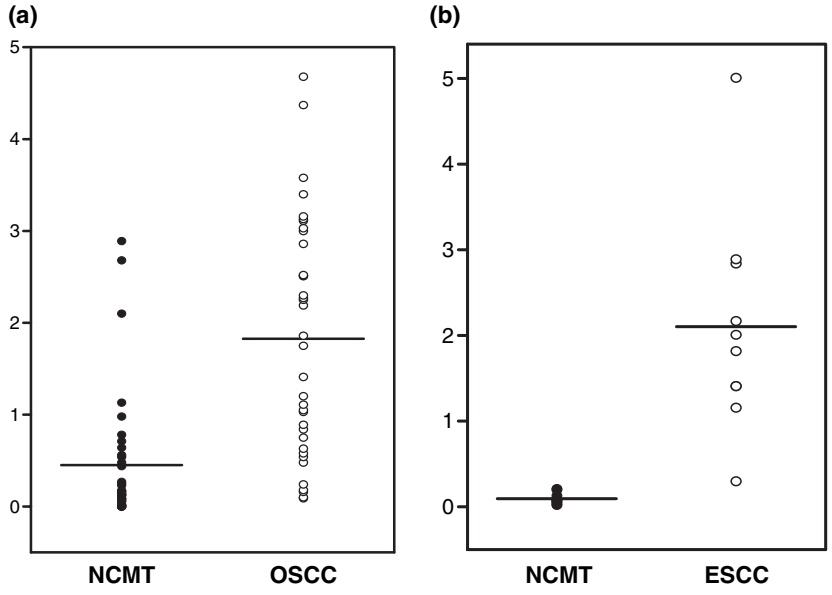

Figure 3 Statistical analyses of *MMP-13* mRNA expression in tissue pairs. (**a**) Oral tissue pairs ($n = 36$). *MMP-13* mRNA expression was 1.83 ± 0.21 and 0.45 ± 0.12 in OSCC and NCMT, respectively ($P < 0.0001$). (**b**) Esophageal tissue pairs ($n = 10$). *MMP-13* mRNA expression was 2.10 ± 0.41 and 0.10 ± 0.02 in ESCC and NCMT, respectively ($P < 0.001$). The expression of *MMP-13* mRNA were normalized with that of *G3PDH*

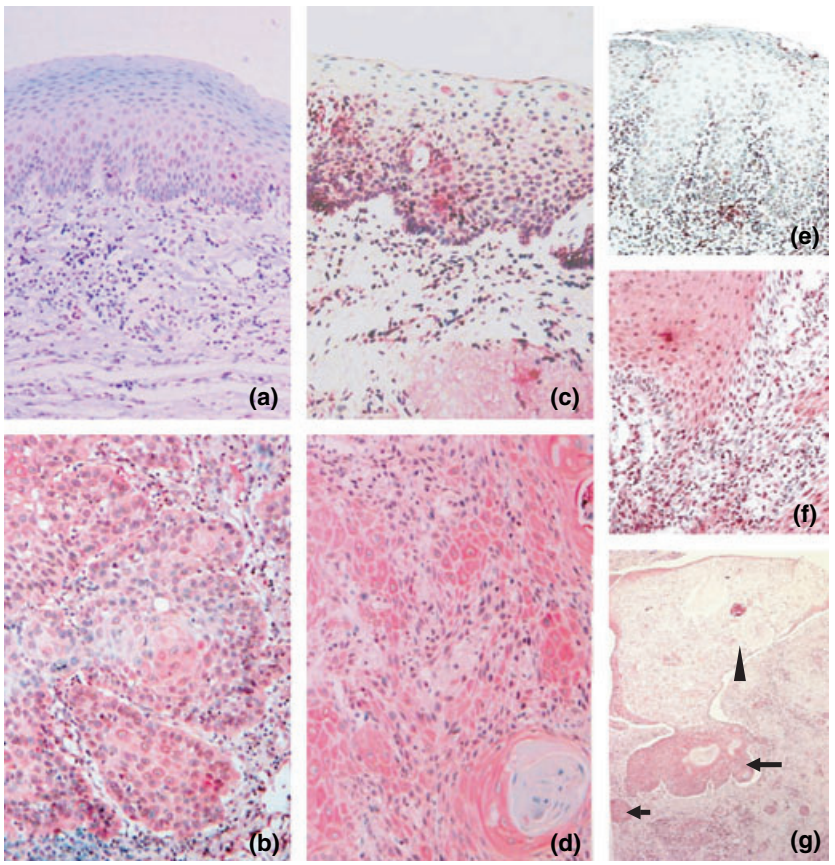

Figure 4 Immunohistochemistry of MMP-13. (**a**) and (**c**) are NCMT. (**b**) and (**d**) are corresponding OSCC of (**a**) and (**c**), respectively; (**e**) and (**f**) are hyperplastic OPL; (**g**), an invasion front. Note the MMP-13 immunoreactivity in stratum spinosum of NCMT. Immunoreactivities were also observed in fibroblasts and inflammatory cells in submucosa. Note the weak and strong nuclear immunoreactivity in addition to cytoplasmic immureactivity in (**a**) and (**f**), respectively. In (**g**), an invasion front (long arrow) and a satellite tumor nest (short arrow) had much stronger MMP-13 immunoreactivity than the non-invasive counterpart (arrow head). Focal accumulation of MMP-13 in extracellular matrix (arrows) was seen in (**c**); (**e**) was scored "–", (**a**) and (**c**) were scored '+' and (**b**), (**d**) and (**f**) were scored '++' in accordance with the criteria described in Materials and Methods. (**a**–**d**, ×200; **e** and **f**, ×100; **g**, ×50)

EGCG treatment with varied dosage at 6, 18 and 24 h. Treatment with >5 or 10 μM EGCG substantially decreased *MMP-13* mRNA expression. Slight *MMP-13* mRNA expression remained in OEC-M1 cells following 20 μM EGCG treatment (Figure 6). Treatment with 20 μM EGCG treatment for 18 h caused most profound suppression of *MMP-13* mRNA expression. MMP-13 protein and activity were analyzed in conditioned media of OEC-M1. Treatment with 0.5–10 μM EGCG only slightly suppressed the MMP-13 protein expression, while treatment with 20 μM markedly suppressed the MMP-13 protein expression for >95% (Figure 7a,c). The gelatin lysis activities of MMP-13 (48 kDa), MMP-2 (62 kDa) and MMP-9 (82 and

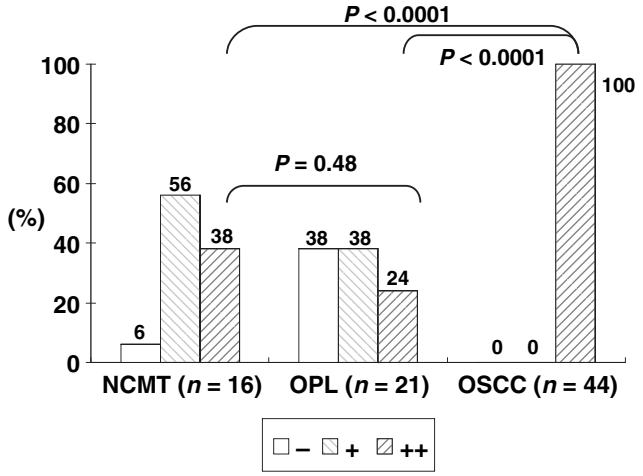

Figure 5 The MMP-13 immunoreactivity during oral carcinogenesis. Note that all OSCC had strong MMP-13 immunoreactivity

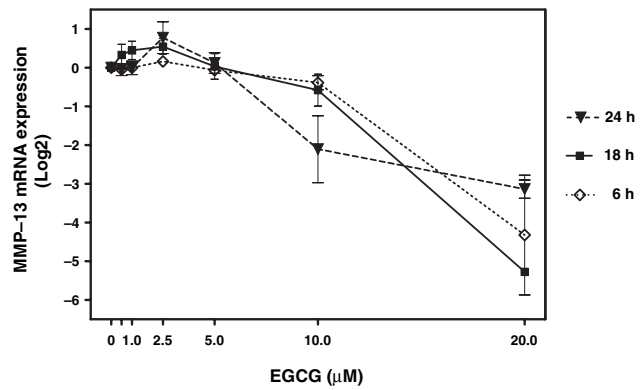

Figure 6 EGCG-modulated suppression of *MMP-13* mRNA expressionin in OEC-M1 cells. OEC-M1 was cultured in FCS-free medium for 24 h. It was then treated with EGCG for 6, 18, and 24 h. *G3PDH* was used as a control. *MMP-13* mRNA expression was normalized with control sample (i.e. 0 μM EGCG/0.2% DMSO). Progressive suppression of *MMP-13* mRNA expression was noted in a dose-dependent manner following treatment with >5 μM EGCG. Twenty micromoles of EGCG treatment for 18 h caused the most profound suppression of *MMP-13* mRNA expression. Experiments were performed in triplicate

92 kDa) were inhibited in a dose-dependent manner following the treatment (Figure 7b,c).

Discussion

MMP-13 has been found to have exceptionally wide substrate specificity when compared with other MMPs (Knauper *et al*, 1997). Therefore, it was believed to be involved in a great diversity of physiological or pathological processes. This study aimed to specify the roles of MMP-13 during oral carcinogenesis. High mRNA expression in OSCC cells in relation to NHOK in our data agrees with the report which indicates that *MMP-13* mRNA was expressed in transformed epidermal keratinocytes and HNSCC cell lines other than primary epidermal keratinocytes (Johansson *et al*, 1997a). Around 90% OSCC tissue had *MMP-13* mRNA

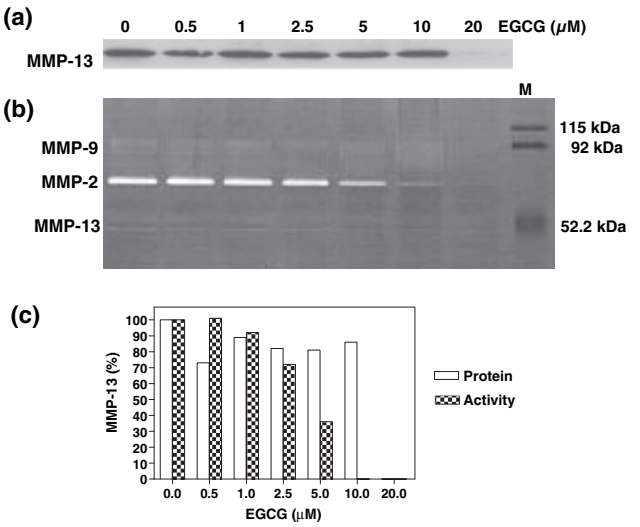

Figure 7 Analysis of conditioned media from OEC-M1 cells treated with EGCG for 18 h. Aliquots of conditioned media for equal number of cultivated cells were analyzed. (**a**) Western blot. The amount of active MMP-13 (48 kDa) was completely diminished following the treatment of 20 μM EGCG. (**b**) Gelatin zymography. Weak zones of degradation at 48 kDa, representing most likely MMP-13 active form, were seen. A progressive decrease of the clear zone following the increase of treatment concentration was noted. Clear zones of active MMP-2 (62 kDa) and relatively fainter MMP-9 bands (92 kDa proform and 82 kDa active form) that attenuated following the EGCG treatment in a dose-dependent manner was seen. (**c**) Quantitative analysis. The relative protein amount and proteolytic activity of MMP-13 are expressed as percentage of controls. Results shown are representative of two independent experiments

expression identified by the present study, which further confirmed the high positive rate in HNSCC (Johansson *et al*, 1997b; Dunne *et al*, 2003). Using tissue pairs, we have identified that OSCC have approximately four times higher *MMP-13* mRNA expression than NCMT. The increase of *MMP-13* mRNA expression in ESCC was also prominent. This study is the first to demonstrate the contrasting *MMP-13* mRNA expression in paired oral tissues. In future, studies of SCC derived from different organs may give further insight into the pathogenetic significance of MMP-13.

The studies on tissues representing multi-stage oral carcinogenesis showed that all OSCC exhibited strong MMP-13 immunoreactivity, significantly higher than that in NCMT or OPL ($<40\%$). As $>60\%$ epithelial dysplasia had strong MMP-13 immunoreactivity, the progressive increase in positive cases with strong MMP-13 immunoreactivity during tumorigenic process has further substantiated the roles of MMP-13 expression in neoplastic genesis (Johansson *et al*, 1997b). It is thus speculated that MMP-13 could serve as a tumor marker of OSCC. NCMT have been related to carcinogens for a long period and the existence of initial cytogenetic defects is credible. Thereby, whether the immunoreactivity of MMP-13 in NCMT is pathologically related or normal physiology is not clear. Recent studies have suggested that the roles of MMP are not only restricted to tumor invasion or metastasis, they also contribute to tumor growth, angiogenesis and migration (McCawley

and Matrisian, 2000; Chang and Werb, 2001). Our findings of progressive increase in MMP-13 expression from NCMT to epithelial dysplasia and OSCC supported this notion. MMP-11 (stromelysin-3) expression was recently found as an early event in oral tumorigenesis which is consistent with our observation (Soni *et al*, 2003). It is presumed that MMP-13 might help cells circumvent adverse environment and survive by acquiring selective advantages for neoplastic formation. Further investigation is underway to specify the role of MMP-13 in acquiring tumor phenotypes.

The findings of *MMP-13* mRNA expression in approximately 90% OSCC have indicated that an increase in transcription could be the main underlying mechanism for MMP-13 up-regulation. All OSCC showing strong MMP-13 immunoreactivity suggested that post-transcriptional modification in translation could be additionally involved in up-regulation. Although a high immunohistochemical intensity of MMP-13 was found in the invasion fronts of OSCC cases, we were unable to highlight the argument that MMP-13 expression can enhance overall cascade of invasion or metastasis, as all OSCC cases exhibited strong MMP-13 immunoreactivity. It is interesting that a certain percentage of epithelial cells had nuclear MMP-13 immunoreactivity. It is unlikely that such nuclear immunoreactivity is non-specific, as our Western blot analysis had detected specific MMP-13 bands. As a wide spectrum of matrix substrate can be digested by MMP-13 (Knauper *et al*, 1997) further studies are required to know whether the unusual localizations of MMP-13 implicate a novel nuclear enzyme activity.

Studies suggest that EGCG suppresses the expression and activity of MMP-2 and MMP-9 (Garbisa *et al*, 2001; Maeda-Yamamoto *et al*, 2003). With the treatment dosage of $>5\ \mu$M, we found that EGCG suppressed *MMP-13* mRNA expression in a dose-dependent manner. The observation of this suppression as soon as 6 h following the treatment suggests that EGCG might regulate MMP-13 directly. Whether EGCG may inhibit the rate of MMP-13 transcription or by post-transcriptional mechanisms which involve reduced RNA stability or processing, or the combination of the two awaits further study. Twenty micromoles of EGCG almost diminished active MMP-13 production. This notable EGCG-mediated suppression of MMP-2 proteolytic activity in this study was compatible with previous studies. It validated the analytical system of our experiments. The banding intensity of MMP-13, although weak, was progressively suppressed by EGCG in a dose-dependent manner, and completely diminished by 10 and 20 μM EGCG. The inhibitory patterns of EGCG on MMP-13 revealed that in a special scenario of concentration, EGCG might exert inhibitory effect on neoplasm progression through suppressing MMP-2, -9 and -13 (Garbisa *et al*, 2001; Maeda-Yamamoto *et al*, 2003). It would be interesting to know what signaling elements are involved in this suppression (Liacini *et al*, 2003). As the increase in MMP-13 is prevalent for oral tumorigenesis, the findings might suggest a role of EGCG in the prevention or therapy of OSCC.

The current study indicated the potential contribution of MMP-13 to early oral carcinogenesis. This MMP-13 may serve as a target for early intervention in oral tumorigenesis. The identification of EGCG for suppressing MMP-13 might further support the application of EGCG in intervening in OSCC.

Acknowledgements

The research was supported by National Research Program for Genomic Medicine grant 91GMP004-3 and National Science Council grant NSC 92-3112-B010-016, Taiwan.

References

Chang C, Werb Z (2001). The many faces of metalloproteases: cell growth, invasion, angiogenesis and metastasis. *Trends Cell Biol* **11:** S37–43.

Chang KW, Kao SY, Tzeng RJ *et al*. (2002). Multiple molecular alterations of FHIT in betel-associated oral carcinoma. *J Pathol* **196:** 300–306.

Curran S, Murray GI (1999). Matrix metalloproteinases in tumour invasion and metastasis. *J Pathol* **189:** 300–308.

Dunne AA, Sesterhenn A, Gerisch A, Teymoortash A, Kuropkat C, Werner JA (2003). Expression of MMP-2, -9 and -13 in cell lines and fresh biopsies of squamous cell carcinomas of the upper aerodigestive tract. *Anticancer Res* **23:** 2233–2239.

Garbisa S, Sartor L, Biggin S, Salvato B, Benelli R, Albini A (2001). Tumor gelatinases and invasion inhibited by the green tea flavanol epigallocatechin-3-gallate. *Cancer* **91:** 822–832.

Johansson N, Westermarck J, Leppa S *et al*. (1997a). Collagenase 3 (matrix metalloproteinase 13) gene expression by HaCaT keratinocytes is enhanced by tumor necrosis factor alpha and transforming growth factor beta. *Cell Growth Differ* **8:** 243–250.

Johansson N, Airola K, Grenman R, Kariniemi AL, Saarialho-Kere U, Kahari VM (1997b). Expression of collagenase-3 (matrix metalloproteinase-13) in squamous cell carcinomas of the head and neck. *Am J Pathol* **151:** 499–508.

Khokha R, Denhardt DT (1989). Matrix metalloproteinases and tissue inhibitor of metalloproteinases: a review of their role in tumorigenesis and tissue invasion. *Invasion Metastasis* **9:** 391–405.

Knauper V, Cowell S, Smith B *et al*. (1997). The role of the C-terminal domain of human collagenase-3 (MMP-13) in the activation of procollagenase-3, substrate specificity, and tissue inhibitor of metalloproteinase interaction. *J Biol Chem* **272:** 7608–7616.

Ko SY, Lin SC, Chang KW *et al*. (2003). Modulation of KGF-1 gene expression in oral fibroblasts by ripe areca nut extract. *J Oral Pathol Med* **32:** 399–407.

Kurahara S, Shinohara M, Ikebe T *et al*. (1999). Expression of MMPS, MT-MMP, and TIMPs in squamous cell carcinoma of the oral cavity: correlations with tumor invasion and metastasis. *Head Neck* **21:** 627–638.

Liacini A, Sylvester J, Li WQ *et al*. (2003). Induction of matrix metalloproteinase-13 gene expression by TNF-alpha is mediated by MAP kinases, AP-1, and NF-kappaB transcription factors in articular chondrocytes. *Exp Cell Res* **288:** 208–217.

Lin SC, Wang CP, Chen YM *et al*. (2002). Regulation of IGFBP-5 expression during tumorigenesis and differentiation of oral keratinocytes. *J Pathol* **198:** 317–325.

Lin SC, Liu CJ, Chiu CP, Chang SM, Lu SY, Chen YJ (2004). Establishment of OC3 oral carcinoma cell line and identification of NF-kappa B activation responses to areca nut extract. *J Oral Pathol Med* **33:** 79–86.

Maeda-Yamamoto M, Suzuki N, Sawai Y *et al*. (2003). Association of suppression of extracellular signal-regulated kinase phosphorylation by epigallocatechin gallate with the reduction of matrix metalloproteinase activities in human fibrosarcoma HT1080 cells. *J Agric Food Chem* **51:** 1858–1863.

McCawley LJ, Matrisian LM (2000). Matrix metalloproteinases: multifunctional contributors to tumor progression. *Mol Med Today* **6:** 149–156.

Soni S, Mathur M, Shukla NK, Deo SV, Ralhan R (2003). Stromelysin-3 expression is an early event in human oral tumorigenesis. *Int J Cancer* **107:** 309–316.

Taniguchi S, Fujiki H, Kobayashi H *et al*. (1992). Effect of (−)-epigallocatechin gallate, the main constituent of green tea, on lung metastasis with mouse B16 melanoma cell lines. *Cancer Lett* **65:** 51–54.

Thomas GT, Lewis MP, Speight PM (1999). Matrix metalloproteinases and oral cancer. *Oral Oncol* **35:** 227–233.

Westermarck J, Kahari VM (1999). Regulation of matrix metalloproteinase expression in tumor invasion. *Faseb J* **13:** 781–792.