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RESEARCH ARTICLE

Matrix metalloproteinases (MMP) 1 and MMP10 but not MMP12 are potential oral cancer markers

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Abstract

The aim of this study was to investigate the mRNA performance of matrix metalloproteinases (MMP) 1, MMP10 and MMP12 as oral cancer markers. With gingiva as the control, the areas under the receiver- operating characteristic curves (AUCs) of the relative gene expressions for MMP1, MMP10 and MMP12 were 0.715, 0.727 and 0.513, respectively. With the margins or neck platysma muscles as controls, the AUCs of MMP1, MMP10 and MMP12 were 0.746 vs 0.626, 0.712 vs 0.683 and 0.697 vs 0.630, respectively. MMP10 displayed the best sensitivity for oral cancer detection with any controls. MMP1 and MMP10 were suitable markers for cancer detection with gingiva and margin as controls. Using neck tissue as the control, only MMP10 was suitable for cancer detection. With margin and neck controls, there were no significant differences for MMP1, MMP10 and MMP12 in different stages, invasion and locations or different habits. Therefore, MMP1 and MMP10 but not MMP12 are potential oral cancer markers.

Keywords: *Oral cancer; matrix metalloproteinases; tumour marker; receiver-operating characteristic curves*

Introduction

Matrix metalloproteinases (MMPs) are synthesized as inactive zymogens (proMMP) and subsequently activated to degrade the extracellular matrix (Nakamura et al. 1998). MMPs may be involved in several steps of cancer development, including tumour growth, differentiation, apoptosis, migration, invasion, angiogenesis and immune response (Egeblad & Werb 2002).

Several array studies found that some of the MMP families are overexpressed in many cancers, including oral cancer and head/neck cancer. For example, MMP1 (Suhr et al. 2007), MMP7, MMP9, MMP12 (Impola et al. 2004) and MMP11 (Soni et al. 2003) have been shown to be overexpressed in oral squamous cell carcinoma (OSCC) studies. MMP1 and MMP10 were overexpressed in head and neck SCC (Kainuma et al. 2006) and MMP1, MMP10 and MMP12 were significantly upregulated in tongue SCCs (Ye et al. 2008).

Among the many MMPs, previous studies suggested that MMP1, MMP10 and MMP12, which are all located in chromosome 11q22.3, may have potential usefulness as predictive markers. MMP10 is known to degrade various components of the extracellular matrix and is inducible in lymphoma cells and accelerates the growth of lymphoid tumours *in vivo* (Van Themsche et al. 2004).

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MMP10 is expressed in cancers of the head, neck, lung (Muller et al. 1991) and oral cavity (Impola et al. 2004). MMP10 can fully activate proMMP-1 (Nicholson et al. 1989); thus, MMP10 is involved in the activation of MMP1 in human cancer tissues. MMP1 is the most highly expressed of the interstitial collagenase-degrading fibrillar collagens, which are major constituents of the extracellular matrix (Kerkela & Saarialho-Kere 2003). MMP12 has been implicated in matrix degradation and macrophage migration (Kerkela & Saarialho-Kere 2003). Both macrophages and transformed epithelial cells may express MMP12 in skin SCC (Kerkela & Saarialho-Kere 2003).

The sample sizes in previous OSCC studies with MMP1 (Suhr et al. 2007), MMP10 and MMP12 (Impola et al. 2004) were limited ($n=15$) and did not include receiver-operating characteristic (ROC) curve analyses (Verdonschot et al. 1993). Previous studies of MMPs, such as MMP10 and MMP12, were not validated by quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) although array data were provided (Impola et al. 2004). Other potential limitations in previous OSCC studies involved the selection of control tissues and the reliability of mining tumour markers. For example, a substantial percentage of light microscopy-negative surgical margin controls from patients with head and neck OSCC were found to contain p53 mutations (Brennan et al. 1995, Partridge et al. 2000). Evidence of p16 promoter methylation within specimens of surgical margins (all 20 tumours on histopathological examination) was found in 11 of 20 cases using the methylation assay (Shaw et al. 2007).

In order to determine the most suitable control tissue, three different kinds of control tissue, including oral safe margin, gingiva and neck platysma muscles, were investigated in the present study. Further, to improve the sample number we used a larger OSCC sample than in previous studies to evaluate the performance of MMP1, MMP10 and MMP12 mRNA quantitation in OSCC detection. The objective of this study was to evaluate the mRNA performance of MMP1, MMP10 and MMP12 as prognostic or predictive markers for oral cancer.

Materials and methods

Tissue samples

This study was approved by the institutional review board at Kaohsiung Medical University. Tissue samples collected included 51 oral tumours, and matched internal control tissue included 25 samples from the oral safe margin and 38 samples from neck platysma muscle. External controls included 11 normal samples of gingival tissue from individuals without malignancy. All these tissue samples were stored at –80°C before use. The pathological diagnosis of all 51 tumour specimens from oral cancer patients was confirmed as OSCC by pathologists and staged according to the TMN system of the American Joint Committee on Cancer (AJCC) classification (Greene et al. 2002). The control tissues were found to be non-malignant under pathological diagnosis. The characteristics of the OSCC tumour samples and the controls are summarized in Table 1.

RNA extraction and quantitative RT-PCR

Total RNA was extracted by Trizol reagents (Invitrogen Corp., Carlsbad, CA, USA) following the manufacturer's manual. The RNA was converted to first strand cDNA using an OmniScript RT kit (Qiagen, Hilden, Germany) and quantitative PCR was performed using iQ SYBR Green Supermix (Bio-Rad Laboratories, Hercules, CA, USA) in an iCycler MyiQ single colour real-time PCR detection system (Bio-Rad). The mRNA levels of MMP1, MMP10 and MMP12 in OSCCs and controls were further validated using quantitative

Table 1. Basic characteristics of patients and tissue samples.

	Tumour	Control		
Variables	OSCC ($n=51$)	Margin[a] ($n=25$)	Neck[a] ($n=38$)	Gingiva[b] ($n=11$)
Age (years), mean (range)	48.5 (28–73)	49.4 (28–73)	49.0 (29–67)	48.2 (24–67)
Gender, *n* (%)				
Male	44 (86.3)	20 (80.0)	32 (84.2)	4 (36.4)
Female	7 (13.7)	5 (20.0)	6 (15.8)	7 (63.6)
Tumour location, *n* (%)				
BM/retromolar area	23 (45.1)	13 (52.0)	16 (42.1)	
Tongue/mouth floor	15 (29.4)	7 (28.0)	12 (31.6)	
Edentulous ridge	9 (17.7)	4 (16.0)	7 (18.4)	
Others (lower lip/ vestibule/soft palate)	4 (7.8)	1 (4.0)	3 (7.9)	
TNM stage, *n* (%)				
I	15 (29.4)	9 (36.0)	10 (26.3)	
II	16 (31.4)	9 (36.0)	12 (31.6)	
III	4 (7.8)	3 (12.0)	2 (5.3)	
IV	16 (31.4)	4 (16.0)	14 (36.8)	
Smoking, *n* (%)				
No	11 (21.6)	5 (20.0)	9 (23.7)	7 (63.6)
Yes	40 (78.4)	20 (80.0)	29 (76.3)	4 (36.4)
Drinking, *n* (%)				
No	11 (21.6)	7 (28.0)	8 (21.1)	10 (90.9)
Yes	40 (78.4)	18 (72.0)	30 (78.9)	1 (9.1)
Betel nut chewing, *n* (%)				
No	7 (13.7)	5 (20.0)	4 (10.5)	9 (81.8)
Yes	44 (86.3)	20 (80.0)	34 (89.5)	2 (18.2)

[a]Margin and neck indicate the oral safe margin and neck platysma muscles, respectively. Samples were collected from some of the patients with oral squamous cell carcinoma (OSCC) for internal controls. [b]Gingiva samples were collected from individuals with no malignancy (rather than the OSCC patients) for external controls.

RT-PCR as previously described (Salani et al. 2007). Primer sets specific for MMP1 (forward: 5′-TGACCTACAGGATTGAAAATTAC-3′, reverse: 5′-TGTAAGTTGTACTCTCTGAAATTG-3′), for MMP10 (forward: 5′-GCATGTTCTGTGACTGAAGAAGA-3′, reverse: 5′-CACATCTATGAAAATACATTCTCTCAC-3′), for MMP12 (forward: 5′-CTCCTTTCATCATACCTCCAATAC-3′, reverse: 5′-CACCATTTCTAACAACCAAACC-3′) and for GAPDH (forward: 5′-CCCTTCATTGACCTCAACTA-3′, reverse: 5′-CCAAAGTTGTCATGGATGAC-3′) were used. The touch-down program was performed as follows: 94°C (1 min); four cycles of 94°C (15 s), 64°C (15 s), 70°C (15 s); four cycles of 94°C (15 s), 61°C (15 s), 70°C (15 s); four cycles of 94°C (15 s), 58°C (15 s), 70°C (15 s); 60 cycles of 94°C for (15 s), 55°C (15 s), 70°C (15 s); 94°C (1 min) and 60°C (5 min). The lengths of the PCR products for MMP1, MMP10, MMP12 and GAPDH were 298, 150, 226 and 401 bp, respectively. All reactions were performed in duplicate. The case/control ratio for MMP1, MMP10 and MMP12 mRNA expression was analyzed using the ΔΔCt method (Livak & Schmittgen 2001). Briefly, the Ct (threshold cycle) value of an MMP gene was subtracted from the Ct value of a reference housekeeping gene, *GAPDH*. Melting curve analyses and electrophoresis were performed to ensure the specificity of the quantitative RT-PCR reactions (Chang et al. 2008).

Statistics

Data analyses were performed using SPSS version 13.0 software. ROC curves (Verdonschot et al. 1993) were used to test the feasibility of using MMP1, MMP10 and MMP12 as predictive tools for detecting oral cancer. Cut-off values for ROC were calculated by 60-ΔCt, where 60 indicates total real-time cycle numbers and no signal was regarded as the Ct value for 60.

Results

To test their predictive performance in OSCC detection, ROC curves of MMP1, MMP10 and MMP12 mRNA expression were constructed for the reference controls of gingiva, margin and neck.

Figure 1A shows that using gingiva as the external control, the AUCs of the relative gene expressions for MMP1, MMP10 and MMP12 were 0.715 (95% confidence interval (CI) 0.585–0.845, $p=0.026$), 0.727 (95% CI 0.594–0.860, $p=0.019$) and 0.513 (95% CI 0.329–0.698, $p=0.890$), respectively.

Figure 1B shows that when safe margins were used as the internal control, the AUCs of the relative gene expression for MMP1, MMP10 and MMP12 were 0.746 (95% CI 0.636–0.855, $p=0.01$), 0.712 (95% CI 0.592–0.833, $p=0.03$) and 0.697 (95% CI 0.575–0.819, $p=0.06$), respectively.

Figure 1C shows that when neck platysma muscles (OSCC/neck) were used as the internal control, the AUCs of the relative gene expression for MMP1, MMP10 and MMP12 were 0.626 (95% CI 0.509–0.743, $p=0.045$), 0.683 (95% CI 0.571–0.795, $p=0.004$) and 0.630 (95% CI 0.513–0.747, $p=0.038$), respectively.

In the analysis with gingiva as the external control, for a specificity of 54.5% the sensitivities of the relative gene expressions for MMP1, MMP10 and MMP2 were 70.6, 78.4, and 58.8 for the cut-off values of 24.43, 39.67 and 25.4, respectively (Table 2). In the analysis with margin as the internal control, for a specificity of 54.2%, the sensitivities of the relative gene expressions for MMP1, MMP10 and MMP12 were 78.4, 78.4 and 68.6, respectively. In the analysis with neck as the internal control, for a specificity of 54.1%, the sensitivities and of MMP1, MMP10 and MMP2 were 54.9, 72.5 and 51.0, respectively.

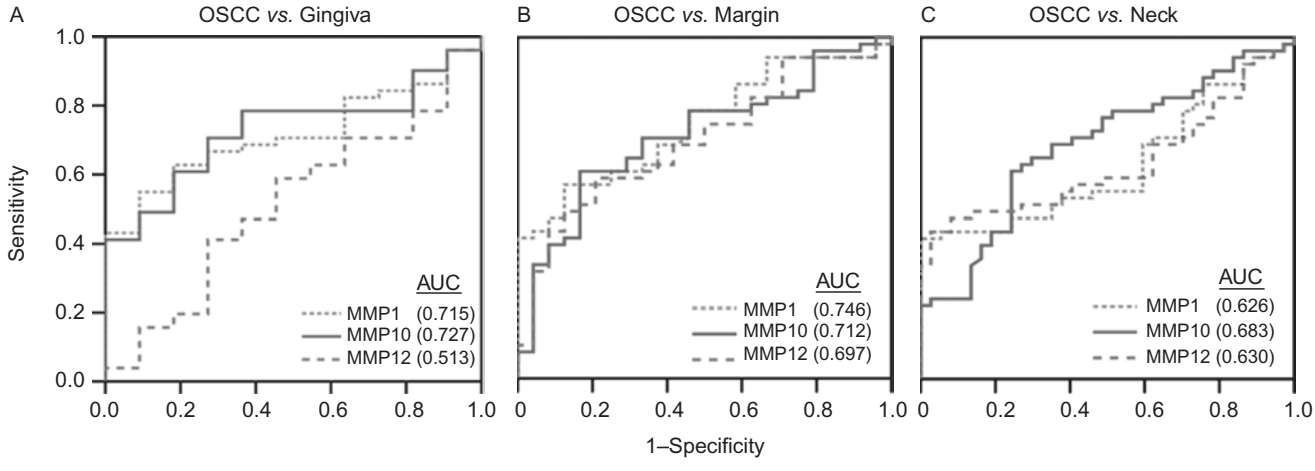

Figure 1. Receiver operating characteristics (ROC) curve analyses of the predictive value of matrix metalloproteinase (MMP) 1, MMP10 and MMP12 for oral cancer. Areas under the curves (AUCs) for MMP1, MMP10 and MMP12 for oral squamous cell carcinoma (OSCC) vs external control (gingiva) or OSCC vs internal controls (margin or neck) are shown.

Analysis of the different specificities listed in Table 2 suggested that the MMP10 had the better sensitivity for OSCC than MMP1 and MMP12 for the reference controls gingiva, margin or neck. While MMP1 and MMP10 showed similar sensitivity for OSCC detection using the reference control of margin, when neck was used as the reference control, MMP10 had a better sensitivity than MMP1 and MMP12.

Table 2. Different cut-offs and their relative sensitivity and specificity for matrix metalloproteinase (MMP) 1, MMP10 and MMP12 in various tissue samples (oral cancer vs three types of control).

	MMP1		MMP10		MMP12	
Specificity (%)	Sensitivity (%)	Cut-off	Sensitivity (%)	Cut-off	Sensitivity (%)	Cut-off
OSCC/gingiva						
54.5	70.6	24.43	78.4	39.67	58.8	25.04
63.6	68.6	24.57	74.5	42.74	47.1	26.30
72.7	66.7	24.64	70.6	51.70	41.2	33.66
OSCC/margin						
54.2	78.4	24.26	78.4	40.53	68.6	24.54
62.5	66.7	24.61	70.6	50.30	62.7	24.67
70.8	60.8	24.71	60.8	54.05	58.8	24.78
OSCC/neck						
54.1	54.9	25.05	72.5	45.57	56.9	25.07
62.2	52.9	25.13	68.6	52.42	52.9	25.20
70.3	47.1	25.38	64.7	53.01	51.0	25.42

OSCC, oral squamous cell carcinoma.

As shown in Table 3, the relative expressions of MMP1, MMP10 and MMP12 were all higher for internal controls of margin or neck compared with gingiva. These results suggest that the overexpression of MMP1, MMP10 and MMP12 is common in OSCC.

With margin as the internal control, the differences in the expressions of MMP1, MMP10 and MMP12 in different stages, and for T of TMN (the greatest tumour diameter/dimension) and different locations were not significant ($p = 0.148$ to 0.975, Kruskal–Wallis test). Similarly, there were no significant differences in MMP1, MMP10 and MMP12 with neck as the internal control ($p = 0.056$ to 0.950, Kruskal–Wallis test) (right side of Table 3).

With margin and neck as the internal controls, the difference of MMP1, MMP10 and MMP12 among

Table 3. Comparison of clinicopathological features and gene expression of matrix metalloproteinase (MMP) 1, MMP10 and MMP12 in oral squamous cell carcinoma (OSCC) and internal controls (margin and neck tissues)[a] and their correlation with patient habits associated with OSCC risk.

	OSCC/margin[a] ($n = 25$)							OSCC/neck[a] ($n = 38$)						
		MMP1		MMP10		MMP12			MMP1		MMP10		MMP12	
	n	Mean	p-Value	Mean	p-Value	Mean	p-Value	n	Mean	p-Value	Mean	p-Value	Mean	p-Value
Clinical data														
Location[b]														
1	13	13.9	0.906[c]	10.4	0.244[c]	15.3	0.171[c]	16	17.2	0.695[c]	15.0	0.072[c]	19.5	0.936[c]
2	7	11.7		14.3		10.1		12	22.3		26.1		20.8	
3	4	13.3		18.0		13.5		7	19.7		19.6		17.4	
4	1	10.0		18.0		1.0		3	20.3		17.0		19.0	
T														
1	9	13.4	0.892[c]	14.3	0.294[c]	11.6	0.712[c]	11	19.8	0.950[c]	22.3	0.050[c]	17.7	0.779[c]
2	11	13.3		14.0		14.3		15	18.8		22.7		19.6	
3 & 4	5	11.6		8.4		12.8		12	20.1		13.0		21.0	
Stage														
I	9	13.4	0.975[c]	21.4	0.148[c]	11.6	0.750[c]	10	21.2	0.783[c]	21.0	0.056[c]	16.0	0.647[c]
II	9	12.8		28.1		14.1		12	17.8		23.8		20.3	
III & IV	7	12.7		26.3		13.4		16	18.7		14.3		19.8	
Habits														
Drinking														
No	7	14.3	0.586[d]	16.6	0.130[d]	16.9	0.102[d]	8	21.8	0.519[d]	18.8	0.830[d]	20.3	0.830[d]
Yes	18	12.5		11.6		11.5		30	18.9		19.7		19.3	
Betel nut chewing														
No	5	12.6	0.892[d]	18.6	0.057[d]	14.8	0.541[d]	4	19.5	1.000[d]	18.8	0.887[d]	16.0	0.505[d]
Yes	20	13.1		11.6		12.6		34	19.5		19.6		19.9	
Smoking														
No	5	16.6	0.221[d]	17.4	0.135[d]	14.4	0.634[d]	9	21.0	0.643[d]	19.1	0.904[d]	22.1	0.420[d]
Yes	20	12.1		11.9		12.7		29	19.0		19.6		18.7	

Mean, the average ΔΔCt value, i.e. (ΔCt of OSCC minus ΔCt of margin) or (ΔCt of OSCC minus ΔCt of neck). T, tumour size and invasiveness in TMN classification.

[a]OSCC/margin indicates the relative gene expressions of MMP1, MMP10 and MMP12 in OSCC compared with that of margin (oral safe margin); OSCC/neck indicates the relative gene expressions of MMP1, MMP10 and MMP12 in OSCC compared with that of neck platysma muscles. [b]Location 1, BM/retromolar area; 2, tongue/mouth floor; 3, edentulous ridge; 4, others (lower lip/vestibule/soft palate). [c]Kruskal–Wallis test. [d]Mann–Whitney test.

patients with different habits, including drinking, betel nut chewing and smoking, were not significant ($p = 0.057$ to 0.892 and $p = 0.519$ to 1.000, respectively, Mann–Whitney test).

Discussion

The ROC results of this study illustrate the importance of identifying suitable controls for determining the predictive value of MMP markers for OSCC. As shown in Figure 1, MMP10 was a suitable OSCC marker when gingival, margin and neck tissues served as controls, with resulting AUCs of 0.727, 0.712 and 0.683, respectively. MMP1 was also a suitable OSCC marker when gingiva and margin served as controls, with AUCs of 0.716 and 0.746, respectively. The AUC for neck as the internal control was 0.630, indicating its lower predictive performance than gingiva and margin. MMP12 was demonstrated to be a suitable OSCC marker when margin was used as the control, with an AUC of 0.697. The AUCs for gingiva and neck were 0.513 and 0.630, respectively, indicating lower predictive performance than for margin as the control. Similar relationships between sensitivity and specificity of these MMPs were also found and are shown in Table 2. These data suggest that different controls may be suitable for certain tumour markers but not for others.

Although moderate p53 gene mutations (Brennan et al. 1995) and p16 promoter methylation (Shaw et al. 2007) were detected in head/neck SCC and OSCC patients with histopathologically negative margins, the margins in our cases are as suitable for the control of MMP1 and MMP10 expression as the gingiva (Figure 1 and Table 2). However, use of the neck platysma muscles as a control in testing the predictive potential of MMP markers for OSCC may encounter problems with tissue-specific expression. Therefore, gingiva and margin seemed to be a suitable control for testing the predictive value of MMP1 and MMP10 for OSCC.

Although the T of the TNM stage was small in this sample of OSCC, no association with stages was detected in the analyses using margin or neck for MMP1, MMP10 or MMP12 detection (Table 3). Although the oral cancer risk was not evaluated in this study, we found that overexpression of MMP1, MMP10 and MMP12 in some oral cancer patients was not associated with the risk factor history of betel quid chewing, cigarette smoking and alcohol consumption (Table 3). The differences of MMP1, MMP10 and MMP12 expression in the tissues of patients with these habits were not significant in this case-matched study using either margin or neck as the control. These results suggest that these MMPs are suitable OSCC markers for most cases.

In conclusion, this study demonstrated that MMP1 and MMP10 but not MMP12 are the potential prognostic or predictive markers for oral cancer.

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References

Brennan JA, Mao L, Hruban RH, Boyle JO, Eby YJ, Koch WM, Goodman SN, Sidransky D. (1995). Molecular assessment of histopathological staging in squamous-cell carcinoma of the head and neck. *N Engl J Med* 332:429–35.

Chang HW, Cheng CA, Gu DL, Chang CC, Su SH, Wen CH, Chou YC, Chou TC, Yao CT, Tsai CL, Cheng CC. (2008). High-throughput avian molecular sexing by SYBR green-based real-time PCR combined with melting curve analysis. *BMC Biotechnol* 8:12.

Egeblad M, Werb Z. (2002). New functions for the matrix metalloproteinases in cancer progression. *Nat Rev Cancer* 2:161–74.

Greene FL, Page DL, Fleming ID, Fritz A, Balch CM, Haller DG, Morrow M(eds.) (2002). *AJCC Cancer Staging Manual*. New York: Springer.

Impola U, Uitto VJ, Hietanen J, Hakkinen L, Zhang L, Larjava H, Isaka K, Saarialho-Kere U. (2004). Differential expression of matrilysin-1 (MMP-7), 92 kD gelatinase (MMP-9), and metalloelastase (MMP-12) in oral verrucous and squamous cell cancer. *J Pathol* 202:14–22.

Kainuma K, Katsuno S, Hashimoto S, Oguchi T, Suzuki N, Asamura K, Usami S. (2006). Differences in the expression of genes between normal tissue and squamous cell carcinomas of head and neck using cancer-related gene cDNA microarray. *Acta Otolaryngol* 126:967–74.

Kerkela E, Saarialho-Kere U. (2003). Matrix metalloproteinases in tumor progression: focus on basal and squamous cell skin cancer. *Exp Dermatol* 12:109–25.

Livak KJ, Schmittgen TD. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods* 25:402–8.

Muller D, Breathnach R, Engelmann A, Millon R, Bronner G, Flesch H, Dumont P, Eber M, Abecassis J. (1991). Expression of collagenase-related metalloproteinase genes in human lung or head and neck tumours. *Int J Cancer* 48:550–6.

Nakamura H, Fujii Y, Ohuchi E, Yamamoto E, Okada Y. (1998). Activation of the precursor of human stromelysin 2 and its interactions with other matrix metalloproteinases. *Eur J Biochem* 253:67–75.

Nicholson R, Murphy G, Breathnach R. (1989). Human and rat malignant-tumor-associated mRNAs encode stromelysin-like metalloproteinases. *Biochemistry* 28:5195–203.

Partridge M, Li SR, Pateromichelakis S, Francis R, Phillips E, Huang XH, Tesfa-Selase F, Langdon JD. (2000). Detection of minimal residual cancer to investigate why oral tumors recur despite seemingly adequate treatment. *Clin Cancer Res* 6:2718–25.

Salani R, Neuberger I, Kurman RJ, Bristow RE, Chang HW, Wang TL, Shih IM. (2007). Expression of extracellular matrix proteins in ovarian serous tumors. *Int J Gynecol Pathol* 26:141–6.

Shaw RJ, Hall GL, Woolgar JA, Lowe D, Rogers SN, Field JK, Liloglou T, Risk JM. (2007). Quantitative methylation analysis of resection margins and lymph nodes in oral squamous cell carcinoma. *Br J Oral Maxillofac Surg* 45:617–22.

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* 107:309–16.

Suhr ML, Dysvik B, Bruland O, Warnakulasuriya S, Amaratunga AN, Jonassen I, Vasstrand EN, Ibrahim SO. (2007). Gene expression profile of oral squamous cell carcinomas from Sri Lankan betel quid users. *Oncol Rep* 18:1061–75.

Van Themsche C, Alain T, Kossakowska AE, Urbanski S, Potworowski EF, St-Pierre Y. (2004). Stromelysin-2 (matrix metalloproteinase 10) is inducible in lymphoma cells and accelerates the growth of lymphoid tumors in vivo. *J Immunol* 173:3605–11.

Verdonschot EH, Wenzel A, Bronkhorst EM. (1993). Applicability of Receiver Operating Characteristic (ROC) analysis on discrete caries depth ratings. *Community Dent Oral Epidemiol* 21:269–72.

Ye H, Yu T, Temam S, Ziober BL, Wang J, Schwartz JL, Mao L, Wong DT, Zhou X. (2008). Transcriptomic dissection of tongue squamous cell carcinoma. *BMC Genomics* 9:69.