Association of –1171 Promoter Polymorphism of Matrix Metalloproteinase-3 with Increased Risk for Oral Cancer

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Abstract. *Background: Matrix metalloproteinase-3 (MMP-3) plays a pivotal role in inflammation and thrombosis. Increased levels of MMP-3 have been correlated with progression of oncogenesis and metastasis. Inflammation-related factors have recently been found to contribute to the development of malignancies. The present study investigated the possible association of –1171 5A/6A polymorphism, which influences expression of MMP-3, with risk for oral cancer. Materials and Methods: This polymorphism was examined by restriction enzyme analysis in 160 patients with oral squamous cell carcinoma (OSCC) and 156 healthy controls of equivalent gender, age and ethnicity (Greeks and Germans). Results: A significant increase of 5A/6A heterozygotes was observed in the whole group and several subgroups of patients compared to controls ($p<0.05$). Nevertheless, in these subgroups and the whole patient group, the 5A allele and carrier frequencies did not differ significantly from those of the control group. The only significant increase of 5A allele-related frequencies ($p<0.05$) in comparison with controls was observed in subgroups of patients with a positive family history of cancer (5A allele carrier frequency only), with advanced stages of cancer (5A allele frequency only) and without positive history of thrombophilia (both 5A allele and carrier frequencies). In addition, the genotypes containing the 5A allele (5A/5A and 5A/6A) had a double risk of OSSC development in smokers (odds ratio (OR) 2.16, 95% confidence interval (CI) 1.04-4.48 and OR 1.78, 95% CI 1.01-3.13, respectively). Conclusion: The results of this study suggest that the high expression 5A allele of the MMP-3 gene is associated with an increased risk for oral cancer in certain individuals.*

Carcinogenesis in the oral region is a multistage process which involves various genetic alterations, such as mutations in oncogenes and tumour suppressor genes, and other factors, such as tobacco and alcohol (1-4). Recent studies incriminate common polymorphisms in genes related to angiogenesis, inflammation and thrombosis with increased risk for oral cancer (5-9). One such factor associated with inflammation, thrombosis and oncogenic signal transduction pathways is matrix metalloproteinase-3 (MMP-3), otherwise known as stromelysin-1, or progelatinase (10, 11).

MMP-3 is a member of a family of structurally related zinc-dependent extracellular proteinases (12). It is synthesized primarily by fibroblasts and to a lesser extent by activated macrophages and keratinocytes adjacent to sites of injury (13). MMP-3 has wide substrate specificity for various extracellular matrix components and therefore it is involved in many biological functions, including extracellular matrix degradation and remodelling, cell proliferation, angiogenesis, as well as induction of synthesis of other metalloproteinases, such as MMP-1 and MMP-9 (14, 15). Normally, MMP-3 expression is low in tissues but it is altered during tumour formation, where remodelling of the extracellular matrix is required (16).

The variety of potential substrates coupled with its widespread distribution suggests that MMP-3 could have extensive effects on tumour progression (16). The expression of MMP-3 in carcinogenesis is regulated primarily at the transcriptional level, but there is also evidence of modulation of mRNA stability in response to growth factors and cytokines secreted by tumour-infiltrating inflammatory cells, as well as by tumour and stromal cells (12, 14, 16). MMP-3 transcription is higher in oral squamous cell carcinoma and several other types of cancer such as lung and breast carcinomas (17-20).

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Key Words: Matrix metalloproteinase-3, oral cancer, angiogenesis, thrombophilia, polymorphism.

Table I. *Prevalence of MMP-3 (–1171 5A/6A) polymorphism in healthy controls and patients with oral cancer (total group of patients and subgroups with regard to cancer stage).*

Genotype	Controls (%)	Patients *P*-value			Patients with cancer stages I&II *P*-value			Patients with cancer stages III&IV *P*-value		
		No. (%)		OR (CI)	No. (%)		OR (CI)	No. (%)		OR (CI)
5A/5A	30 (19.2%)	36 (22.5%)	N.S.	1.99 (0.98-4.05)	20 (22.7%)	N.S.	1.86 (0.81-4.26)	16 (22.2%)	N.S.	2.46 (0.99-6.84)
6A/6A	51 (32.7%)	40 (25%)		1 (referent)	26 (29.5%)		1 (referent)	14 (19.4%)		1 (referent)
6A/5A	75 (48.1%)	84 (53.5%)	<0.05	1.65 (0.95-2.85)	42 (47.7%)	N.S.	1.35 (0.71-2.58)	42 (58.3%)	<0.05	2.13 (1.02-4.45)
Total	156 (100%)	160 (100%)			88 (100%)			72 (100%)		
Prevalence of 5A allele										
5A allele frequency	43.3%	48.8%	N.S.		46.6%	N.S.		51.4%	N.S.	
Carrier frequency of 5A allele	67.3%	75%	N.S.		70.5%	N.S.		80.6%	<0.05	

Fischer's *p*-value; N.S.: *p*-value not significant; OR: odds ratio; CI: confidence interval (95%).

At position –1171 of the promoter region of the *MMP-3* gene, a polymorphism of 5 or 6 adenosines (5A/6A) affects its transcription (20). The 5A allele results in higher gene expression in fibroblasts and vascular smooth muscle cells compared to the 6A allele, which possibly is recognized better by *trans* acting elements such as transcription repressors (20). The 5A allele is more abundant in Europeans (frequency range 40-50%) than East Asians (frequency range 7-20%) (17, 20-27).

The presence of the 5A allele has been associated with susceptibility for coronary heart disease and abdominal aneurysm, inflammatory disorders such as areca-related oral submucous fibrosis, celiac disease as well as ovarian and breast carcinomas (24, 26, 28, 29). On the other hand, the lower expression-associated 6A allele has been associated with increased risk for colorectal and lung cancer (25, 27, 30).

In this study the 5A/6A polymorphism in the *MMP-3* gene in patients with oral cancer and healthy controls was examined in order to determine whether it is associated with increased risk for this type of cancer.

Materials and Methods

The individuals under study were 316 unrelated Greeks and Germans; 160 patients with oral squamous cell carcinoma and 156 healthy blood donors of similar age, gender and ethnicity. The patients were mostly men (N=128) and their age ranged between 40-84 years (58.6±10.1 years, mean 58.6 years). The gender ratio of the controls (N=114 men) and their age (ranged 31-83 years; 54.4±11.7 years, mean 54.4 years) were comparable to those of the patients.

The patients who were included in this study had developed oral cancer and were operated recently or up to a decade ago. In addition to clinical presentation, a biopsy with pathological diagnosis of tumour stages I-IV and a family history regarding cancer and thrombophilia were available. Sixty of them (37.5%) had one or two first degree relatives with cancer and their age range (median=58.7 years) did not differ significantly from the whole group of patients. Furthermore, 32 patients (20%) had one or two first-degree relatives with idiopathic thrombosis and an earlier age range (median=58 years) but again with no statistical difference compared to the whole group. Sixteen patients (10%) had a positive family history for both cancer and thrombophilia (median age=56.3 years).

Most of the participants in the two groups generally worked in a low-risk environment (with the exception of one patient and three controls who worked in chemical factories). No data were available on controls regarding their family history or smoking and alcohol consumption habits.

Blood samples were collected from patients and controls under study after informed consent has been obtained. DNA was isolated from blood with the use of NucleoSpin™ kit (Macherey-Nagel GmbH & Co, Dóren, Germany). Molecular detection of the (–1171 5A/6A) polymorphism in the *MMP-3* gene was performed by restriction fragment length polymorphism typing. This involved a combination of PCR amplification and digestion with restriction endonuclease *Thr111I* followed by gel electrophoretic analysis. The PCR conditions consisted of an initial denaturation step at 95˚C, followed by 30 cycles of 94˚C for 55 s, 65˚C for 1 min, and 72˚C for 55 s, as well as a final elongation step at 72˚C for 5 min. The primers used were forward: 5'-GGTTCTCCATTCCTTTGATGGGGGGAAAGA-3' and reverse:

Table II. *Prevalence of MMP-3 (-1171 5A/6A) polymorphism in healthy controls and patients with oral cancer with regard to family history of either cancer or thrombophilia.*

Genotype	Controls (%)	Family history of cancer						Family history of thrombophilia					
		Patients with			Patients without			Patients with			Patients without		
		(%)	*P*-value	OR	(%)	*P*-value	OR	(%)	*P*-value	OR	(%)	*P*-value	OR
5A/5A	30 (19.2%)	22 (36.7%)	<0.05	2.86 (1.23-6.7)	14 (14%)	N.S.	1.51 (0.61-3.75)	6 (18.8%)	N.S	0.78 (0.26-2.31)	30 (23.4%)	<0.05	3.27 (1.44-7.43)
6A/6A	51 (32.7%)	14 (23.3%)		1 (referent)	26 (26%)		1 (referent)	14 (43.8%)		1 (referent)	26 (20.3%)		1 (referent)
6A/5A	75 (48.1%)	24 (40%)	N.S.	1.13 (0.53-2.43)	60 (56%)	<0.05	1.99 (1.05-3.8)	12 (37.5%)	N.S.	0.59 (0.25-1.39)	72 (56.3%)	<0.05	2.32 (1.22-4.39)
Total	156 (100%)	60 (100%)			100 (100%)			32 (100%)			128 (100%)		
Prevalence of 5A allele													
5A allele frequency	43.3%	56.7%	<0.05		44%	N.S.		37.5%	N.S.		51.6%	<0.05	
Carrier frequency of 5A allele	67.3%	76.7%	N.S.		74%	N.S.		56.3%	N.S.		79.7%	<0.05	

Fischer's *p*-value; N.S.: *p*-value not significant; OR: Odds ratio; CI: confidence interval (95%).

5'-CTTCCTGGAATTCACATCACTGCCACCACT-3'. After digestion, the products were separated on a 3% agarose gel and stained with ethidium bromide. As a result, the 5A alleles were represented by DNA bands of 97 and 32 bp and the 6A alleles by a DNA band of 129 bp, whereas the heterozygotes displayed a combination of both alleles (129, 97 and 32 bp).

The frequencies of alleles and genotypes of the whole group or subgroups of patients were compared to the respective frequencies of the control group using the Chi-square or Fisher's exact test and odds ratios. The significance level was set at $p<0.05$ and the results are presented as hazard ratios with 95% confidence intervals (CI). For the purpose of statistical analysis all unknown variables of controls were assumed to be nil, thus, odds ratios obtained for some subgroups of patients may overestimate the true likehood of MMP-3 genotypes and those variables. Statistical analyses were carried out using SAS® software (version 9.0; SAS Worldwide Headquarters SAS Institute Inc., Cary, NC, USA).

Results

The prevalence of detected MMP-3 genotypes in healthy controls and patients with oral cancer are shown in Tables I-III. The data for the two tested populations (Greeks and Germans) were analyzed together since there were no significant differences of allele frequencies among the two subgroups of healthy controls. The observed frequency of the 5A allele in the control group was 43.3%, similar to other European populations. All 5A/6A genotype distributions were as expected according to Hardy-Weinberg equilibrium in the control group, as well in the group of patients.

The comparison between controls and patients showed that although the number/frequency of 5A/6A heterozygotes were increased in patients, their 5A allele and carrier frequencies did not differ significantly from those of the control group (Table I). The same pattern was also observed in subgroups of patients in regard to their smoking, drinking habits (Table III) and with no family history of cancer (Table II). Interestingly, in patients with a positive family history of cancer, an increased percentage of 5A/5A homozygotes was observed, therefore the 5A allele frequency was also significantly elevated in comparison with that in the controls ($p<0.05$, Table II).

In patients without a positive family history of thrombophilia, all genotypes as well as the 5A allele and carrier frequencies were significantly different from controls. In this subgroup, compared to individuals with the 6A/6A genotype, the relative risk for oral squamous cell carcinoma for 6A/5A heterozygotes was double (OR 2.32, 95% CI 1.22-4.39), while for 5A/5A homozygotes was triple (OR 3.27, 95% CI 1.44-7.43). Patients with a positive family history of thrombophilia had no statistical difference with controls, but the sample was rather small to evaluate these findings (Table II).

No significant difference from controls was observed in patients with early stages of cancer. On the contrary, patients with advanced cancer stages had a significant increase in 5A allele carrier frequency compared to controls ($p<0.05$, Table I).

Table III. *Prevalence of MMP-3 (-1171 5A/6A) polymorphism in healthy controls and patients with oral cancer with regard to either alcohol consumption or smoking habits.*

Genotypes	Controls (%)	Tobacco abuse						Alcohol abuse					
		Patients with			Patients without			Patients with			Patients without		
		(%)	*P*-value	OR	(%)	*P*-value	OR	(%)	*P*-value	OR	(%)	*P*-value	OR
5A/5A	30 (19.2%)	34 (22.7%)	$p<0.05$	2.16 (1.04-4.48)	2 (20%)	N.S.	1.18 (0.2-7.09)	12 (23.1%)	N.S.	2.08 (0.75-5.79)	24 (22%)	N.S.	1.99 (0.91-4.38)
6A/6A	51 (32.7%)	36 (24%)		1 (referent)	4 (40%)		1 (referent)	14 (26.9%)		1 (referent)	26 (24.1%)		1 (referent)
6A/5A	75 (48.1%)	80 (53.3%)	$p<0.05$	1.78 (1.01-3.13)	4 (40%)	N.S.	0.67 (0.14-3.08)	26 (50%)	N.S.	1.48 (0.66-3.33)	58 (53.7%)	$p<0.05$	1.71 (0.93-3.16)
Total	156 (100%)	150 (100%)			10 (100%)			52 (100%)			108 (100%)		
Prevalence of 5A allele													
5A allele Frequency	43.3%	49.3%	N.S.		40%	N.S.		48.1%	N.S.		49.1%	N.S.	
Carrier Frequency of 5A allele	67.3%	76%	N.S.		60%	N.S.		73.1%	N.S.		75.9%	N.S.	

Fischer's *p*-value; N.S.: *p*-value not significant; OR: odds ratio; CI: confidence interval (95%).

Discussion

MMP-3 exhibits several activities that could render it a particularly good tumour promoter. In addition to degrading numerous extracellular-matrix components, MMP-3 may activate gelatinase B and other collagenases, as well as release several cell surface molecules, including E-cadherin, a known contributor to cancer development (31).

Compared to normal tissues, MMP-3 levels are significantly higher in several tumours, including 42.6-88.5% of oral squamous cell carcinoma (OSCC) (16, 18, 30, 32). A recent study of cDNA microarray analysis showed that *MMP-3* mRNA levels are up to 15 times higher in OSCC samples compared to normal oral mucosa (33). Furthermore, some studies report an association of the high expression 5A allele of *MMP-3* with breast, pulmonary, ovarian and colorectal cancer (25, 29).

In this study the genotypes and allele frequencies of the -1171 5A/6A polymorphism were investigated in a cohort of 160 patients with oral cancer in comparison to 156 healthy controls of equivalent age, gender and ethnicity. Despite the relatively small sample of studied individuals, the overall data obtained revealed a minor association of the –1171 5A/6A polymorphism with an increased risk for OSCC in a subset of studied patients. Compared to the controls, a significant increase of 5A/6A heterozygotes was observed in the whole group and several subgroups of patients. Nevertheless, in these subgroups and the whole patient group the 5A allele and carrier frequencies did not differ significantly from those of the control group. The only significant increase of 5A allele-related frequencies, in comparison to controls, was observed in subgroups of patients with a positive family history of cancer (5A allele carrier frequency only), with advanced stages of cancer (5A allele frequency only) and without a positive family history of thrombophilia (both 5A allele and carrier frequencies).

Despite the relatively small sample size, several interesting conclusions may be drawn based on the results of this study. The fact that significantly increased prevalence of the 5A allele was observed only in patients without a positive family history of thrombophilia and not in those with a positive one, suggests that the 5A allele is probably not related to thrombosis. To our knowledge, there is no study investigating the possible involvement of MMP-3 with thrombophilia.

Another interesting observation is that the high expression 5A allele frequency is increased in the subgroup of patients with a positive family history of cancer and not in the subgroup without it. These results probably reflect the important association of the –1171 5A/6A polymorphism with other types of cancer, such as ovarian and breast carcinomas (25, 29).

Interestingly, the statistical analysis showed that 5A/6A heterozygotes have a greater relative risk for developing oral cancer of advanced stages than of early ones. This result could be explained by the fact that tumour aggressiveness is related to the increase of invasiveness and metastases (30, 32). High *MMP-3* expression levels have

been correlated with progression of oncogenesis and especially with invasive OSCC (30-34). It is also known that MMP-3 levels increase in OSCC tissues with lymph node metastasis compared to OSCCs without metastasis (33). Therefore, it can be assumed that the high expression 5A allele of the –1171 promoter polymorphism is associated with a more aggressive type of oral cancer.

In addition, this study revealed that the genotypes containing the 5A allele (5A/5A and 5A/6A) may double the risk of OSSC development in smokers (Table III). A similar observation has been reported regarding the combined effect of the 5A allele and smoking on the elevated risk of non-small cell lung carcinoma (17). One possible explanation is that smoking induces alteration of the extracellular matrix by increasing mRNA levels of MMPs and tissue inhibitors of metalloproteinases (35). Therefore, the effect of polymorphisms affecting expression of *MMP* genes in smokers may depend upon the balance between MMPs and tissue inhibitors of metalloproteases (27). Certainly, the role of MMP-3 in smokers needs to be verified by further studies with a larger sample size.

In conclusion, this study did reveal a minor association of increased *MMP-3* expression with OSSC in certain individuals. The fact that MMP-3 is but one of MMPs present in the oral region might explain its limited contribution to cancer risk. Indeed, a similar minor association of the *MMP-1* 1G/2G polymorphism with oral oncogenesis has been found in previous studies by our group and another (36, 37). The relative order of the MMP gene cluster on 11q22 is centromere-MMP-10-MMP-1-MMP-3-MMP-13-telomere (38). Therefore, haplotype analysis for *MMP-3* and the adjacent *MMP-1* locus was performed in order to investigate whether, in our sample, these two polymorphisms are in linkage disequilibrium. The haplotype analysis revealed no linkage disequilibrium between *MMP-1* and *MMP-3* polymorphisms in either controls or patients. Interestingly, the results obtained from two other studies performed with French and Japanese populations, revealed moderate ($p=0.41$ and $p=0.46$) and significant ($p=0.88$ and $p=0.75$) linkage disequilibrium in cases and controls, respectively (37, 38). Additional haplotype analysis in functional polymorphisms of the two remaining MMPs in this gene cluster will probably bring more insight into the complex relation between the polymorphisms in *MMP* genes and cancer risk (18, 19).

It is of great importance to perform further genetic association studies regarding the contribution of additional MMPs or other inflammation and thrombosis related factors to oncogenesis in the oral region. Any positive findings could ultimately result in the undertaking of preventive measures safeguarding the health status and lives of certain at risk individuals in the general population.

Acknowledgements

This work was co-funded by the European Social Fund and National Resources (EPEAEK II "Pythagoras" 70/3/7391) grant to E.V.

References

1 Hanahan D and Weinberg RA: The hallmarks of cancer. Cell *100*: 57-70, 2000.

2 Vokes EE, Weichselbaum RR, Lippman SM and Hong WK: Head and neck cancer. N Engl J Med *328*: 184-194, 1993.

3 Muscat JE and Wynder EL: Tobacco, alcohol, asbestos and occupational risk factors for laryngeal cancer. Cancer 69: 2244-2251, 1992.

4 Jefferies SA and Foulkes WD: Review. Genetic mechanisms in squamous cell carcinoma of the head and neck. Oral Oncol *37*: 115-126, 2001.

5 Song C, Xing D, Tan W, Wei Q and Lin D: Methylenetetrahydrofolate reductase polymorphisms increase risk of esophageal squamous cell carcinoma in a Chinese population. Cancer Res *61*: 3272-3275, 2001.

6 Vairaktaris E, Yapijakis C, Wiltfang JV, Ries J, Vylliotis A, Derka S, Vasiliou S and Neukam FW: Are factor V and prothrombin mutations associated with increased risk of oral cancer? Anticancer Res *25*: 2561-2566, 2005.

7 Vairaktaris E, Yapijakis C, Kessler P, Vylliotis A, Ries J, Wiltfang J, Vassiliou S, Derka S and Neukam FW: Methylenetetrahydrofolate reductase polymorphism and minor increase of risk for oral cancer. J Cancer Res Clin Oncol *132*: 219-222, 2006.

8 Vairaktaris E, Yapijakis C, Serefoglou Z, Vylliotis A, Ries J, Nkenke E, Wiltfang J, Derka S, Vassiliou S, Springer I, Kessler P and Neukman FW: Plasminogen activator inhibitor-1 polymorphism is associated with increased risk for oral cancer. Oral Oncol *42*: 888-892, 2006.

9 Vairaktaris E, Yapijakis C, Derka S, Vassiliou S, Serefoglou Z, Vylliotis A, Wiltfang J, Springer I, Nkenke E, Kessler P and Neukam FW: Association of platelet glycoprotein Ia polymorphism with minor increase of risk for oral cancer. Eur J Surg Oncol *32*: 455-457, 2006.

10 Galis ZS, Sukhova GK, Lark MW and Libby P: Increased expression of matrix metalloproteinases and matrix degrading activity in vulnerable regions of human atherosclerotic plaques. J Clin Invest *94*: 2493-2503, 1994.

11 Kerkelä E and Saarialho-Kere U: Review Article. Matrix metalloproteinases in tumor progression: focus on basal and squamous cell skin cancer. Exp Dermatol *12*: 109-125, 2003.

12 Matrisian LM: Cancer biology: extracellular proteinases in malignancy. Curr Biol *9*: 776-778, 1999.

13 Nagase H and Woessner JF: Matrix Metalloproteinases. J Biol Chem *274*: 21491-21494, 1999.

14 Kahari VM and Saarialho-Kere U: Matrix metalloproteinases and their inhibitors in tumour growth and invasion. Ann Med *31*: 34-45, 1999.

15 Sage EH, Reed M, Funk SE, Truong T, Steadele M, Puolakkainen P, Maurice DH and Bassuk JA: Cleavage of the matricellular protein SPARC by matrix metalloproteinase 3 produces polypeptides that influence angiogenesis. J Biol Chem *278*: 37849-37857, 2003.

16 Johanssona N, Ahonenb M and Kähäri VM: Matrix metalloproteinases in tumor invasion. Cell Mol Life Sc *57*: 5-15, 2000.
17 Fang S, Jin X, Wang R, Li Y, Guo W, Wang N, Wang Y, Wen D, Wei L and Zhang J: Polymorphisms in the MMP1 and MMP3 promoter and non-small cell lung carcinoma in North China. Carcinogenesis *26*: 481-486, 2005.
18 Ylipalosaari M: Matrix metalloproteinases (MMPs) in oral carcinomas. Dissertation, Oulu University Press, Oulu 2005.
19 Thomas GT, Lewis MP and Speight PM: Matrix metalloproteinases and oral cancer. Oral Oncol *35*: 227-233, 1999.
20 Ye S, Eriksson P, Hamsten A, Kurkinen M, Humphries SE and Henney AM: Progression of coronary atherosclerosis is associated with a common genetic variant of the human stromelysin-1 promoter which results in reduced gene expression, J Biol Chem *271*: 13055-13060, 1996.
21 Ghilardi G, Biondi M L, Caputo M, Leviti S, Monti M and Guagnellini E: A single nucleotide polymorphism in the matrix metalloproteinase-promoter enhances breast cancer susceptibility. Clin Cancer Res *8*: 3820-3823, 2002.
22 Lei H, Zaloudik J and Vorechovky I: Lack of association of the -1171 5A allele of the MMP3 promoter with breast cancer. Clin Chem *5*: 798-799, 2002.
23 Dörr S, Lechtenböhmer N, Rau R, Herborn G, Wagner U, Müller-Myhsok B, Hansmann I and Keyszer G: Association of a specific haplotype across the genes MMP1 and MMP3 with radiographic joint destruction in rheumatoid arthritis. Arthritis Res Ther *6*: 199-207, 2004.
24 Mora B, Bonamico M, Ferri M, Megiorni F, Osborn J, Pizzuti A and Mazzilli MC: Association of the matrix metalloproteinase-3 (MMP-3) promoter polymorphism with celiac disease in male subjects. Hum Immunol *66*: 717-720, 2005.
25 Hinoda Y, Okayama N, Takano N, Fujimura K, Suehiro Y, Hamanaka Y, Hazama S, Kitamura Y, Kamatani N and Oka M: Association of functional polymorphisms of matrix metalloproteinase-1 (MMP-1) and MMP-3 genes with colorectal cancer. Int J Cancer *102*: 526-529, 2002.
26 Tu HF, Liu CJ, Chang CS, Lui MT, Kao SY, Chang CP and Liu TY: The functional –1171 (5A/6A) polymorphisms of matrix metalloproteinase 3 gene as a risk factor for oral submucous fibrosis among male areca users. Oral Pathol Med *35*: 99-103, 2006.
27 Su L, Zhou W, Asomaning K, Lin X, Wain JC, Lynch TJ, Liu G and Christiani DC: Genotypes and haplotypes of matrix metalloproteinase 1, 3 and 12 genes and the risk of lung cancer Carcinogenesis *27*: 1024-1029, 2006.
28 Yoon S, Tromb G, Vongpunsawad S, Ronkainen A, Juvonen T and Kuivaniemi H: Genetic analysis of MMP3 and MMP9 and PAI-1 in Finnish patients with abdominal aortic or intracranial aneurysms. Biochem Biophys Res Communic *265*: 563-568, 1999.
29 Biondi ML, Turri O, Leviti S, Seminati R, Cecchini F, Bernini M, Ghilardi G and Guagnellini E: MMP1 and MMP3 polymorphisms in promoter regions and cancer. Clin Chem *46*: 2023-2024, 2000.
30 Kusukawa J, Sasaguri Y, Mormatsu M and Kameyama T: Expression of matrix metalloproteinase-3 in stage I and II squamous cell carcinoma of the oral cavity. J Oral Maxillofac Surg *53*: 530-534, 1995.
31 McCawley L, Crawford H, King L, Mudgett J and Matrisian L: A protective role for matrix metalloproteinase-3 in squamous cell carcinoma. Cancer Res *64*: 6965-6972, 2004.
32 Kurahara S, Shinohara M, Ikebe T, Nakamura S, Beppu M, Hiraki A, Takeuchi H and Shirasuna K: 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, 1999.
33 Nagata M, Fujita H, Ida H, Hoshina H, Inoue T, Seki Y, Ohnishi M, Ohyama T, Shingaki S, Kaji M, Saku T and Takagi R: Identification of potential biomarkers of lymph node metastasis in oral squamous cell carcinoma by cDNA microarray analysis. Int J Cancer *106*: 683-689, 2003.
34 Kusukawa J ,Harada H, Shima I, Sasaguri Y, Kameyama T and Mormatsu M: The significance of the epidermal growth factor receptor and matrix metalloproteinase -3 in squamous cell carcinoma of the oral cavity. Eur J Cancer B Oral Oncol *32*: 217-221, 1996.
35 Yin L, Morita A and Tsuji T: Alterations of extracellular matrix induced by tobacco smoke extract. Arch Dermatol Res *292*: 188-194, 2000.
36 Vairaktaris E, Yapijakis C, Derka S, Serefoglou Z, Vassiliou S, Nkenke E, Ragos V, Vylliotis A, Spyridonidou S, Tsigris C, Yannopoulos A, Tesseromatis C, Neukam FW and Patsouris E: Minor association of matrix metalloproteinase-1 (–1607 1G/2G) polymorphism with increased risk for oral squamous cell carcinoma. Anticancer Res *27*: 459-464, 2006.
37 Zinzindohoue F, Blons H, Hans S, Loriot MA, Houllier AM, Brasnu D, Laccourreye O, Tregouet DA, Stucker I and Laurent-Puig P: Single nucleotide polymorphisms in MMP1 and MMP3 gene promoters as risk factor in head and neck squamous cell carcinoma. Anticancer Res *24*: 2021-2026, 2004.
38 Pendas AM, Matilla T Estivill X and Lopez-Otin C: The human collagenase-3 (CLG3) gene is located on chromosome 11q22.3 clustered to other members of the matrix metalloproteinase gene family. Genomics *26*: 615-618, 1995.
39 Hinoda Y, Okayama N, Takano N, Fujimura K, Suehiro Y, Hamanaka Y, Hazama S, Kitamura Y, Kamatani N and Oka M: Association of functional polymorphisms of matrix metalloproteinase (MMP)-1 and MMP-3 genes with colorectal cancer. Int J Cancer *102*: 526-529, 2002.

Received May 16, 2007
Revised July 20, 2007
Accepted August 30, 2007