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Natriuretic peptide receptor A is related to the expression of vascular endothelial growth factors A and C, and is associated with the invasion potential of tongue squamous cell carcinoma

Y. Nakao[1], S. Yamada[2], S. Yanamoto[1], T. Tomioka[2], T. Naruse[1], T. Ikeda[3], H. Kurita[2], M. Umeda[1]

[1]Department of Clinical Oral Oncology, Unit of Translational Medicine, Course of Medical and Dental Sciences, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan; [2]Department of Dentistry and Oral Surgery, Shinshu University School of Medicine, Matsumoto, Japan; [3]Department of Oral Pathology and Bone Metabolism, Unit of Basic Medical Sciences, Course of Medical and Dental Sciences, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

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Abstract. Natriuretic peptide receptor A (NPRA) is one of the natriuretic peptide receptors. NPRA has been reported to play a role in the carcinogenesis of various tumours, as well as functional roles in renal, cardiovascular, endocrine, and skeletal homeostasis. The clinicopathological significance of NPRA in tongue squamous cell carcinoma (TSCC) was examined in this study. The overexpression of NPRA was more frequent in TSCC (21/58, 36.2%) than in the normal oral epithelium (0/10, 0%) ($P < 0.05$). It was also more frequently observed in cancers with higher grades according to the pattern of invasion (grades 1–2 vs. grades 3–4, $P < 0.01$). Additionally, there was a tendency towards an association between the N classification and NPRA expression (N0 vs. N1–2, $P = 0.06$). Significant correlations were also observed between the expression of NPRA and that of VEGF-A ($P < 0.001$) and VEGF-C ($P < 0.001$). The high-NPRA expression group had a significantly poorer prognosis, with a 5-year disease-specific survival rate of 39.7%, compared to 97.0% in the low-expression group ($P < 0.001$). Multivariate analysis suggested that the overexpression of NPRA may also be an independent prognostic factor ($P < 0.05$). In conclusion, NPRA is associated with VEGF expression levels, invasion, and metastasis, and may be a prognostic factor in TSCC patients.

Key words: NPRA; invasion; metastasis; migration; tongue squamous cell carcinoma.

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Oral squamous cell carcinoma (OSCC) is the most common malignant tumour in the head and neck region and accounts for more than 90% of cancers in the oral cavity[1]. The oral tongue is the most common site of OSCC. The primary therapeutic modality for OSCC is surgery.

Although recent advances in surgical techniques and anticancer agents have improved tumour regression and survival for patients with OSCC, the wide surgical resection of OSCC inevitably causes various oral dysfunctions. Therefore, new treatment strategies are urgently needed.

The presence of neck lymph node metastasis is strongly related to a poor prognosis in squamous cell carcinoma of the head and neck[2–4]. Moreover, previous studies have reported that an alteration in the expression of adhesion-related molecules is associated with a poor prognosis in OSCC patients[5–8]. Several tissue and biological markers have been identified as possible indicators of tumour aggressiveness and metastatic capability[9].

Angiogenesis and lymphangiogenesis are also crucial for tumour progression and nodal metastasis in OSCC[10]. Some of the main angiogenic and lymphangiogenic factors identified belong to the vascular endothelial growth factor (VEGF) family of ligands and receptors, and include the angiogenic factors VEGF-A and VEGF receptor 2 (VEGFR2), as well as the lymphangiogenic factors VEGF-C/VEGF-D and VEGFR3[11,12].

Natriuretic peptide receptor A (NPRA) is one of the natriuretic peptide receptors; it is a membrane-bound guanylate cyclase that serves as the receptor for both atrial and brain natriuretic peptides (ANP and BNP, respectively)[13]. NPRA synthesizes the intracellular second-messenger cyclic guanosine monophosphate (cGMP) and activates cGMP-dependent protein kinase (PKG) in response to ANP binding[14]. The expression of NPRA in cells of inflamed and injured tissues and in tumours has been reported[15,16]. NPRA has also been shown to have effects on the cardiovascular system, including natriuretic, diuretic, vasorelaxant, and anti-proliferative responses altering the intracellular levels of cGMP[17,18]. Furthermore, it affects cell growth, proliferation, apoptosis, and inflammation through cGMP-regulated transcription factors, ion channels, phosphodiesterases, and possibly other effector proteins[19–21]. Increases in blood pressure and hypertensive heart disease have been shown in NPRA-gene knockout mice[22]. More recently, NPRA has been reported to play a role in the carcinogenesis of various tumours, as well as functional roles in renal, cardiovascular, endocrine, and skeletal homeostasis[15,23,24]. Moreover, the expression of VEGF was found to be down-regulated in the lungs of NPRA-deficient mice when compared to wild-type mice[15]. However, the relationships between the expression of NPRA and clinicopathological features, as well as between the expression of NPRA and VEGF, have not yet been investigated in tongue squamous cell carcinoma (TSCC).

The purpose of this study was to determine the clinicopathological significance of NPRA in TSCC and clarify its correlation with VEGF expression in TSCC. An immunohistochemical analysis was performed to determine the relationships between the expression of NPRA and clinicopathological features in clinical TSCC samples.

Materials and methods

Patients

The study protocol was approved by the Ethics Committee of Nagasaki University Graduate School of Biomedical Sciences. Paraffin-embedded sections were obtained from the biopsy specimens of 58 patients with TSCC who had undergone radical surgery in Nagasaki University Hospital. The tumour stage was classified according to the TNM classification of the Union for International Cancer Control, and the histological differentiation was defined according to the World Health Organization classification. The pattern of invasion was determined according to the classification of Yamamoto et al.[25]. As controls, 10 samples of the normal oral epithelium were obtained from 10 patients undergoing the routine surgical removal of third molars; informed consent was obtained from these patients.

Immunohistochemical staining and evaluations

Serial 4-μm-thick specimens were taken from tissue blocks. The sections were deparaffinized in xylene, soaked in target retrieval solution buffer (Dako, Glostrup, Denmark), and placed in an autoclave at 121 °C for 5 min for antigen retrieval. Endogenous peroxidase was blocked by incubating sections with 0.3% H_2O_2 in methanol for 30 min. Immunohistochemical staining was performed using the Envision system (Envision+; Dako, Carpinteria, CA, USA). The primary antibodies used were directed against NPRA (ab70848; Abcam, Cambridge, UK), VEGF-A, and VEGF-C (Santa Cruz Biotechnology, Inc., Dallas, TX, USA). Sections were incubated with the primary antibody overnight at 4 °C. Reaction products were visualized by immersing the sections in diaminobenzidine (DAB) solution, and the samples were counterstained with Meyer's haematoxylin and then mounted. Negative controls were prepared by replacing the primary antibody with phosphate-buffered saline.

The immunoreactivity of NPRA was scored based on the staining intensity and immunoreactive cell percentage as follows[24]. The percentage of immunoreactive cells was graded on a scale of 0 to 4: score 0 for $\leq$5% positive tumour cells, score 1 for 6–25% positive tumour cells, score 2 for 26–49% positive tumour cells, score 3 for 50–75% positive tumour cells, and score 4 for $\geq$76% positive tumour cells. The staining intensity was graded from 0 to 3: 0 for no staining, 1 for weak staining (light yellow), 2 for moderate staining (yellow–brown), and 3 for strong staining (brown). The final score was obtained by multiplying the quality and intensity scores. A final score of 0 was considered negative, of 1–3 was regarded as weakly positive, and of 4–8 was regarded as strongly positive. In this study, strongly positive staining of NPRA was defined as the overexpression of this molecule.

In accordance with a previous study on VEGF expression[26], proportional scores described the estimated fraction of positively stained tumour cells as follows: staining index 0 = no staining, 1 = <10% of tumour cells, 2 = 10–50% of tumour cells, 3 = 50–80% of tumour cells, and 4 = >80% of tumour cells. The intensity score represented the estimated staining intensity as follows: staining index 0 = no staining, 1 = weak staining, 2 = moderate staining, and 3 = strong staining. The immunohistochemical overexpression of VEGF-A and VEGF-C was defined as a total score greater than 4[26]. Total score is defined as the sum of scores of staining index and intensity scores.

Statistical analysis

Statistical analyses were performed using StatMate III (ATMS Co., Tokyo, Japan). The relationships between the expression of NPRA and clinicopathological features were assessed using Fisher's exact test.

Continuous data are presented as the mean ± standard deviation. Datasets were examined by one-way analysis of variance (ANOVA) followed by Scheffé's post-hoc test. A survival analysis was performed with Kaplan–Meier curves and related log-rank tests. Prognostic factors were assessed using the Cox proportional hazards model. *P*-values of less than 0.05 were considered significant.

Results

Relationships between NPRA expression and clinicopathological features

Immunohistochemistry with an anti-NPRA polyclonal antibody was performed on samples obtained from 58 patients with TSCC. Representative immunohistochemical staining results are shown in Fig. 1A and B. The overexpression of NPRA was undetectable in the normal epithelium. NPRA staining was mainly detected in the cytoplasm of squamous cell carcinoma cells (Fig. 1B). The nuclei of tumours were also partially stained. The overexpression of NPRA was more frequent in TSCC (21/58, 36.2%) than in the normal oral epithelium (0/10, 0%) ($P < 0.05$). It was also more frequently observed in cancers of higher grades according to the pattern of invasion grades 1–3 vs. grade 4C/4D, $P < 0.01$; Table 1). Additionally, there was a tendency towards an association between the N classification and NPRA expression (N0 vs. N1–2, $P = 0.06$). These results strongly suggest that the overexpression of NPRA might be a strong predictor of survival through invasive potential in TSCC patients.

Correlation between the expression of NPRA and VEGFs in TSCC

Angiogenesis and lymphangiogenesis have been shown to play crucial roles in tumour progression and nodal metastasis in OSCC[10]. The family of VEGFs, including VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, placental growth factor, and VEGF-F, has previously been reported as crucially involved in angiogenesis and lymphangiogenesis[27]. Of these VEGFs, VEGF-A and VEGF-C expression levels have previously been correlated with lymph node metastasis in oesophageal squamous cell carcinoma[28]. In the present study, the relationships between the expression of NPRA and the expression of VEGF-A and VEGF-C were examined. Immunohistochemical staining of VEGF-A and VEGF-C was detected in the cytoplasm of both normal tissue and tumour cells (Fig. 1C and D). These proteins were found to be strongly expressed at the invasion front of the tumour. The overexpression of VEGF-A was more frequent in TSCC (28/58, 48.3%) than in the normal oral epithelium (0/10, 0%) ($P < 0.01$). In addition, the overexpression of VEGF-C was more frequent in TSCC (21/58, 36.2%) than in the normal oral epithelium (0/10, 0%) ($P < 0.01$). Correlations were also observed between the expression of NPRA and that of VEGF-A and VEGF-C (VEGF-A, $P < 0.001$; VEGF-C, $P < 0.001$; Table 2). These results also strongly suggest that the overexpression of NPRA might be a strong predictor of survival.

Relationship between NPRA expression and survival analysis

The 5-year disease-specific survival rates of TSCC patients according to NPRA expression were plotted (Fig. 2). The high-NPRA expression group had a significantly poorer prognosis, with a 5-year disease-specific survival rate of 39.7%, compared to 97.0% in the low-expression group ($P < 0.001$). This result also strong-

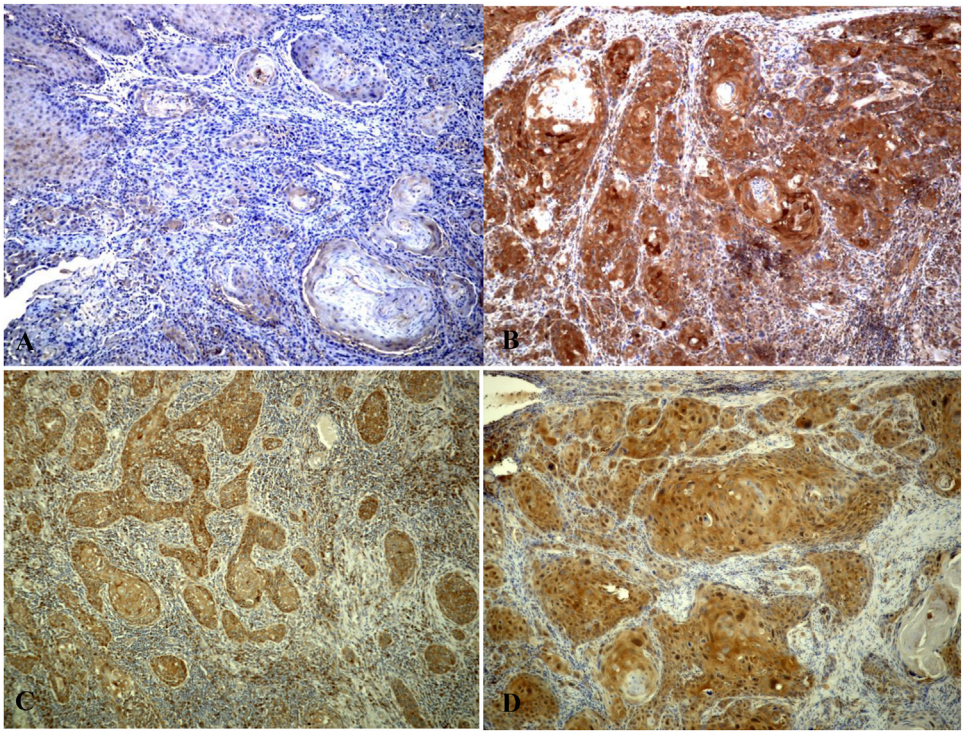

Fig. 1. Representative immunohistochemical staining for natriuretic peptide receptor A (NPRA) in tongue squamous cell carcinoma. (A) Immunohistochemical staining for NPRA demonstrating the negative expression of NPRA2 (×100). (B) Immunohistochemical staining for NPRA demonstrating the strong cytoplasmic and partial nuclear expression of NPRA (×100). (C) Immunohistochemical staining for vascular endothelial growth factor A (VEGF-A) demonstrating the strong cytoplasmic expression of VEGF-A (×100). (D) Immunohistochemical staining for vascular endothelial growth factor C (VEGF-C) demonstrating the strong cytoplasmic expression of VEGF-C (×100).

Table 1. Relationships between the overexpression of NPRA and clinicopathological features.

Characteristics	Number of samples	NPRA overexpression (−)	NPRA overexpression (+)	*P*-value
Normal epithelia	10	10	0	<0.05
Squamous cell carcinoma	58	37	21	
Sex				
Male	40	26	14	NS
Female	18	11	7	0.776
Age (years)				
≤63	29	19	10	NS
>63	29	18	11	0.785
T classification				
T1 + T2	51	34	17	NS
T3 + T4	7	3	4	0.219
N classification				
N0	44	31	13	NS
N1 + N2	14	6	8	0.061
Stage				
Stage I–II	43	30	13	NS
Stage III–IV	15	7	8	0.109
Differentiation				
Well-differentiated	52	35	17	NS
Moderately/poorly differentiated	6	2	4	0.101
Pattern of invasion				
Grades 1–3	48	35	13	<0.01
Grades 4C/4D	10	2	8	
Local recurrence				
Negative	50	34	16	NS
Positive	8	3	5	0.095
Secondary metastasis				
Negative	43	30	13	NS
Positive	15	7	8	0.109

NS, not significant.

Table 2. Relationships between the overexpression of NPRA and VEGF-A and VEGF-C expression.

Characteristics	Number of samples	NPRA overexpression negative	NPRA overexpression positive	*P*-value
VEGF-A expression				
Negative	30	26	4	<0.001
Positive	28	11	17	
VEGF-C expression				
Negative	37	32	5	<0.001
Positive	21	5	16	

NPRA, natriuretic peptide receptor A; VEFG, vascular endothelial growth factor.

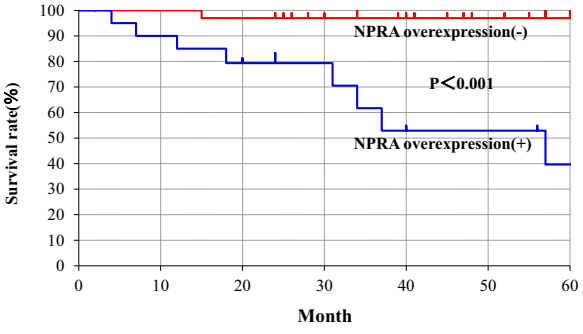

Fig. 2. Kaplan–Meier curves for the analysis of 5-year disease-specific survival. The 5-year overall survival rates according to NPRA expression were plotted for tongue squamous cell carcinoma patients. The high-NPRA expression group had a significantly poorer prognosis than the low-NPRA expression group ($P < 0.001$).

ly suggests that the overexpression of NPRA might be a strong predictor of survival, similar to the clinicopathological features described above.

For the purpose of examining the relationships between the expression of NPRA and clinicopathological features, univariate analysis (log-rank test) and multivariate analysis (Cox proportional hazards model) were performed with factors showing significant correlations with NPRA overexpression. The univariate analysis revealed that the prognosis of TSCC patients could be predicted by the pattern of invasion (grade 1–3 vs. 4, $P < 0.001$; Table 3), NPRA overexpression (NPRA overexpression negative vs. positive, $P < 0.01$), and VEGF-A overexpression (VEGF-A overexpression negative vs. positive, $P < 0.001$). Multivariate analysis also suggested that the overexpression of NPRA may be an independent prognostic factor (NPRA overexpression negative vs. positive, $P < 0.05$). These results also strongly suggest that the overexpression of NPRA might be a potent predictor of survival, similar to the clinicopathological features described above.

Discussion

Several recent studies have reported the clinicopathological and functional significance of NPRA in various cancers. The role of NPRA in cancer has been described by Kong and colleagues using animal models and small interfering RNA (siRNA)[15]. They reported that NPRA attenuation or deficiency protects from tumourigenesis in lung and ovarian cancers and melanomas by several mechanisms, including decreasing local inflammation, controlling the expression of the tumour suppressor gene *Rb*, and blocking VEGF expression[15]. The ectopic expression of a plasmid encoding NP73–102 (the NH2-terminal peptide of the atrial natriuretic peptide prohormone comprising residues 73 to 102) was found to down-regulate NPRA expression and also to inhibit the activation of the proinflammatory transcription factors nuclear factor kappa B (NF-κB), activator protein 1, and Erk-1 and -2 in human bronchial epithelial adenocarcinoma A549 cells[15]. In oesophageal squamous cell carcinoma, Zhao et al. reported that NPRA expression was strongly detected in the cytoplasm, but was undetectable or very weak in the nucleus, and that the expression of NPRA was associated with histological differentiation, TNM stage, and a poor prognosis[24]. In TSCC, NPRA expression was also

Table 3. Univariate and multivariate analysis of different prognostic parameters.

Characteristics	Univariate analysis			Multivariate analysis		
	HR	95% CI	*P*-value	HR	95% CI	*P*-value
Pattern of invasion (1–3 vs. 4C, 4D)	12.469	13.258–597.560	<0.001	2.290	0.880–13.496	NS (0.360)
VEGF-A overexpression (negative vs. positive)	9.736	1.583–21.971	<0.01	0.604	0.120–3.040	NS (0.541)
VEGF-C overexpression (negative vs. positive)	2.393	0.649–10.227	NS (0.179)	–	–	–
NPRA overexpression (negative vs. positive)	19.384	4.387–79.156	<0.001	10.410	1.636–66.252	<0.05

HR, hazard ratio; CI, confidence interval; NS, not significant; VEFG, vascular endothelial growth factor; NPRA, natriuretic peptide receptor A.

detected mainly in the cytoplasm, with partial staining in the nuclei also seen. The NPRA staining pattern of TSCC was similar to that of oesophageal squamous cell carcinoma. However, the staining pattern of NPRA in other tumours and its significance are uncertain. Therefore, further studies are needed to confirm the significance of the cytoplasmic staining of NPRA in TSCC. In this study, NPRA overexpression was significantly associated with the pattern of invasion. Additionally, there was a tendency towards an association between the N classification and NPRA overexpression. These findings suggest that NPRA expression may enhance the invasion and metastasis potentials in TSCC. Additionally, the analysis of the disease-specific survival rate in this study revealed a poor prognosis in the NPRA overexpression group in TSCC patients.

On multivariate analysis, NPRA expression was also a significant independent prognostic factor. The above findings suggest that, as seen in oesophageal squamous cell carcinoma[24], NPRA may affect the prognosis through invasion potential and lymph node metastasis in TSCC patients.

In a functional analysis of NPRA, tumour angiogenesis was reported to result from NPRA-induced activation of the VEGF/stromal-derived factor 1α (SDF-1α)/chemokine (C–X–C motif) receptor 4 (CXCR4) axis and the subsequent recruitment of stem cell progenitors to form a reactive stroma that could interact with the tumour cells and promote tumour growth[29]. In the analysis of an oesophageal squamous cell carcinoma cell line, NPRA was reported to promote migration and invasive potentials through the regulation of matrix metalloproteinase (MMP)-2 and MMP-9 activation[24]. However, in this study, NPRA expression was significantly associated with the pattern of invasion and revealed a tendency towards an association with the N classification. Based on these findings, NPRA expression may have crucial roles in the invasion and metastasis potentials in TSCC. However, the precise mechanisms by which NPRA acts on the migration and invasive potentials of TSCC remain uncertain. In addition, since the roles of NPRA in oral carcinogenesis remain uncertain, further examination of NPRA in oral carcinogenesis, including dysplastic lesions, is needed.

Angiogenesis and lymphangiogenesis are known to be crucial for tumour progression and nodal metastasis in OSCC[10]. The family of VEGFs, including VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, placental growth factor, and VEGF-F, play crucial roles in angiogenesis and lymphangiogenesis[28]. Of these, the expression levels of VEGF-A and VEGF-C have been correlated with lymph node metastasis in oesophageal squamous cell carcinoma[28]. Naruse et al. reported that VEGF-A and VEGF-C might be related to tumour growth and invasion, respectively[26]. In the present study, it was found that the expression of NPRA was correlated with the expression of VEGF-A and VEGF-C. According to the previous reports described above, NPRA regulates VEGF expression in some malignant tumours[15,29]. However, the association between NPRA and VEGF expression in TSCC remains unknown. In this study, NPRA expression was significantly associated with both VEGF-A and VEGF-C expression. The results of this study suggest that NPRA may play a pivotal role in tumour invasion through VEGF signalling. However, further studies are needed to clarify the precise relationships between NPRA–VEGF signalling, tumour proliferation, and invasion potential.

A strength of this study is that it reports the significance of NPRA expression in TSCC, including the clinicopathological significance and the association with VEGF-A and VEGF-C expression. Limitations of this study are that the investigation was based on a single primary site tumour at one institution and histopathological analysis. Therefore, further investigations are needed based on other sites of oral cancer and the molecular and biological analysis of NPRA.

In conclusion, these promising data indicate that NPRA is associated with VEGF expression levels, invasion, and metastasis, and might be a prognostic factor in TSCC patients. Further studies on the expression and function of NPRA may offer additional indicators for the diagnosis and treatment of TSCC patients.

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Competing interests

None declared.

Ethical approval

The study protocol was approved by the Ethics Committee of Nagasaki University Graduate School of Biomedical Sciences (No. 14120447).

Patient consent

Not required.

References

1. Johnson NW, Jayasekara D, Amarasinghe AA. Squamous cell carcinoma and precursor lesions of the oral cavity: epidemiology and aetiology. *Periodontology 2000* 2011;**57**:19–37.
2. Hicks Jr WL, North Jr JH, Loree TR, Maamoun S, Mullins A, Orner JB, Bakamjian VY, Shedd DP. Surgery as a single modality therapy for squamous cell carcinoma of the oral tongue. *Am J Otolaryngol* 1998;**19**:24–8.
3. Sessions DG, Spector GJ, Lenox J, Parriott S, Haughey B, Chao C, Marks J, Perez C. Analysis of treatment results for base of

tongue cancer. *Laryngoscope* 2003;**113**:1252–61.
4. González-García R, Naval-Gías L, Rodríguez-Campo FJ, Sastre-Pérez J, Muñoz-Guerra MF, Gil-Díez Usandizaga JL. Contralateral lymph neck node metastasis of squamous cell carcinoma of the oral cavity: a retrospective analytic study in 315 patients. *J Oral Maxillofac Surg* 2008;**66**:1390–8.
5. Ziober BL, Silverman Jr SS, Kramer RH. Adhesive mechanisms regulating invasion and metastasis in oral cancer. *Crit Rev Oral Biol Med* 2001;**12**:499–510.
6. Bánkfalvi A, Krassort M, Buchwalow IB, Végh A, Felszeghy E, Piffkó J. Gains and losses of adhesion molecules (CD44, E-cadherin, and beta-catenin) during oral carcinogenesis and tumour progression. *J Pathol* 2002;**198**:343–51.
7. Arora S, Kaur J, Sharma C, Mathur M, Bahadur S, Shukla NK, Deo SV, Ralhan R. Stromelysin 3, Ets-1, and vascular endothelial growth factor expression in oral precancerous and cancerous lesions: correlation with microvessel density, progression, and prognosis. *Clin Cancer Res* 2005;**11**:2272–84.
8. Yanamoto S, Kawasaki G, Yoshitomi I, Iwamoto T, Hirata K, Mizuno A. Clinicopathologic significance of EpCAM expression in squamous cell carcinoma of the tongue and its possibility as a potential target for tongue cancer gene therapy. *Oral Oncol* 2007;**43**:869–77.
9. Brinkman BM, Wong DT. Disease mechanism and biomarkers of oral squamous cell carcinoma. *Curr Opin Oncol* 2006;**18**: 228–33.
10. Sasahira T, Kirita T, Kurihara M, Yamamoto K, Bhawal UK, Bosserhoff AK. MIA-dependent angiogenesis and lymphangiogenesis are closely associated with progression, nodal metastasis and poor prognosis in tongue squamous cell carcinoma. *Eur J Cancer* 2010;**46**:2285–94.
11. Kopfstein L, Veikkola T, Djonov VG, Baeriswyl V, Schomber T, Strittmatter K, Stacker SA, Achen MG, Alitalo K, Christofori G. Distinct roles of vascular endothelial growth factor-D in lymphangiogenesis and metastasis. *Am J Pathol* 2007;**170**:1348–61.
12. Lohela M, Bry M, Tammela T, Alitalo K. VEGFs and receptors involved in angiogenesis versus lymphangiogenesis. *Curr Opin Cell Biol* 2009;**21**:154–65.
13. Chinkers M, Garbers DL, Chang MS, Lowe DG, Chin HM, Goeddel DV, Schulz S. A membrane form of guanylate cyclase is an atrial natriuretic peptide receptor. *Nature* 1989;**338**:78–83.
14. Pandey KN, Nguyen HT, Li M, Boyle JW. Natriuretic peptide receptor-A negatively regulates mitogen-activated protein kinase and proliferation of mesangial cells: role of cGMP-dependent protein kinase. *Biochem Biophys Res Commun* 2000;**271**:374–9.
15. Kong X, Wang X, Xu W, Behera S, Hellermann G, Kumar A, Lockey RF, Mohapatra S, Mohapatra SS. Natriuretic peptide receptor A as a novel anticancer target. *Cancer Res* 2008;**68**:249–56.
16. Wang X, Raulji P, Mohapatra SS, Patel R, Hellermann G, Kong X, Vera PL, Meyer-Siegler KL, Coppola D, Mohapatra S. Natriuretic peptide receptor A as a novel target for prostate cancer. *Mol Cancer* 2011;**10**:56. http://dx.doi.org/10.1186/1476-4598-10-56.
17. Currie MG, Geller DM, Cole BR, Boylan JG, YuSheng W, Holmberg SW, Needleman P. Bioactive cardiac substances: potent vasorelaxant activity in mammalian atria. *Science* 1983;**221**:71–3.
18. de Bold AJ, Borenstein HB, Veress AT, Sonnenberg H. A rapid and potent natriuretic response to intravenous injection of atrial myocardial extract in rats. Reprinted from Life Sci. 28: 89–94, 1981. *J Am Soc Nephrol* 2001;**12**:403–9.
19. Pedram A, Razandi M, Kehrl J, Levin ER. Natriuretic peptides inhibit G protein activation. Mediation through cross-talk between cyclic GMP-dependent protein kinase and regulators of G protein-signaling proteins. *J Biol Chem* 2000;**275**:7365–72.
20. Silberbach M, Roberts Jr CT. Natriuretic peptide signalling: molecular and cellular pathways to growth regulation. *Cell Signal* 2001;**13**:221–31.
21. Fiscus RR. Involvement of cyclic GMP and protein kinase G in the regulation of apoptosis and survival in neural cells. *Neurosignals* 2002;**11**:175–90.
22. Oliver PM, Fox JE, Kim R, Rockman HA, Kim HS, Reddick RL, Pandey KN, Milgram SL, Smithies O, Maeda N. Hypertension, cardiac hypertrophy, and sudden death in mice lacking natriuretic peptide receptor A. *Proc Natl Acad Sci U S A* 1997;**94**:14730–5.
23. Pandey KN. Internalization and trafficking of guanylyl cyclase/natriuretic peptide receptor-A. *Peptides* 2005;**26**:985–1000.
24. Zhao Z, Liu H, Yang Y, Sun K, Li M, Zhang J, Cai H, Wang J. Expression of natriuretic peptide receptor-A in esophageal squamous cell carcinomas and the relationship with tumor invasion and migration. *World J Surg Oncol* 2014;**12**:154. http://dx.doi.org/10.1186/1477-7819-12-154.
25. Yamamoto E, Kohama G, Sunakawa H, Iwai M, Hiratsuka H. Mode of invasion, bleomycin sensitivity, and clinical course in squamous cell carcinoma of the oral cavity. *Cancer* 1981;**51**:2175–80.
26. Naruse T, Kawasaki G, Yanamoto S, Mizuno A, Umeda M. Immunohistochemical study of VEGF expression in oral squamous cell carcinomas: correlation with the mTOR-HIF-1α pathway. *Anticancer Res* 2011;**31**:4429–37.
27. Otrock ZK, Makarem JA, Shamseddine AI. Vascular endothelial growth factor family of ligands and receptors: review. *Blood Cells Mol Dis* 2007;**38**:258–68.
28. Ding MX, Lin XQ, Fu XY, Zhang N, Li JC. Expression of vascular endothelial growth factor-C and angiogenesis in esophageal squamous cell carcinoma. *World J Gastroenterol* 2006;**12**:4582–5.
29. Mallela J, Ravi S, Jean Louis F, Mulaney B, Cheung M, Sree Garapati U, Chinnasamy V, Wang C, Nagaraj S, Mohapatra SS, Mohapatra S. Natriuretic peptide receptor A signaling regulates stem cell recruitment and angiogenesis: a model to study linkage between inflammation and tumorigenesis. *Stem Cells* 2013;**31**:1321–9.

Address:
Shin-ichi Yamada
Department of Dentistry and Oral Surgery
Shinshu University School of Medicine
3-1-1
Asahi
Matsumoto 390-8621
Japan
Tel: +81 (0)263 37 2675; Fax: +81 (0)263 37 2676
E-mail: yshinshin@shinshu-u.ac.jp