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Prediction of outcome of patients with oral squamous cell carcinoma using vascular invasion and the strongly positive expression of vascular endothelial growth factors

Sachiko Seki [a], Mutsunori Fujiwara [b], Masaaki Matsuura [c], Shuichi Fujita [a], Hisazumi Ikeda [d], Izumi Asahina [d], Tohru Ikeda [a,*]

[a] Department of Oral Pathology and Bone Metabolism, Nagasaki University Graduate School of Biomedical Sciences, Japan
[b] Department of Clinical Pathology, Japanese Red Cross Medical Center, Japan
[c] Bioinformatics Group, Genome Center, Japanese Foundation for Cancer Research, Japan
[d] Department of Regenerative Oral Surgery, Nagasaki University Graduate School of Biomedical Sciences, Japan

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SUMMARY

Vascular invasion and lymph node metastasis have been used as histopathological prognosticators of cancers including oral squamous cell carcinoma (OSCC). In addition to metastatic potential via blood vessels, tumor-induced angiogenesis might also be associated with prognosis. However, the efficacy of combined evaluation of vascular invasion and angiogenesis-associated molecules for the prognosis of OSCC remains obscure. This is also the case in lymph node metastasis and lymphovasculogenesis-associated molecules. The aim of this study was to examine factors related to prognosis to improve the accuracy of prognostic prediction of OSCC using vasculogenesis-associated markers. Ninety specimens of patients from 1991 to 2002 with previously untreated OSCC, who underwent either biopsy or surgery, were histopathologically and immunohistochemically analyzed using antibodies for vascular endothelial growth factor (VEGF)-A, VEGF-C, cyclooxygenase (COX)-2 and Midkine. The ninety cases were composed of 72 well-differentiated, 12 moderately differentiated and 6 poorly differentiated OSCC. Efficient models of prognostic prediction were evaluated by extensive statistical analyses. The presence of vascular invasion or lymph node metastasis was confirmed to be significantly associated with poor prognosis in the univariate analysis. Multivariate logic regression analysis suggested that patients with the strongly positive expression of either VEGF-A or VEGF-C had a significant association with poor prognosis even in patients without vascular invasion and in early-stage patients. Neither COX-2 nor Midkine contributed to predict the prognosis of the patients. The strongly positive expression of VEGF-A or VEGF-C was suggested to reinforce the histopathological diagnosis of vascular invasion and improve the accuracy and efficacy of prognostic prediction of OSCC.

Introduction

The incidence of oral squamous cell carcinoma (OSCC) is relatively high and it has been reported to be the eighth most common cancer worldwide.[1,2] In addition, increasing incidence of OSCC is observed more in Europe, Japan, and in the younger generation in many western countries.[3]

Despite advances in clinical treatments, survival rates of OSCC have not improved for decades.[4] It is considered particularly important to find and treat OSCC at an early stage. In the oral cavity, OSCC is most frequently seen in the posterior-lateral border of the tongue.[1,5]

VEGF-A, which was discovered as a factor that induces capillary and endothelial cell growth, was also recognized to be associated with tumor-induced angiogenesis.[6] VEGF-C, one of the other members of the VEGF family, was found to be preferentially associated with tumor-induced lymphovasculogenesis.[7,8]

COX-2 is a well-known enzyme that metabolizes arachidonic acid and induces cell membrane phospholipid-derived inflammation. Close association of the expression of COX-2 with carcinogenesis and tumor-induced angiogenesis has begun to be elucidated[9–11] and the expression of COX-2 has been identified as a possible marker to recognize carcinogenesis.[12]

MK, which was initially found as a molecule expressed in embryonal carcinoma cells, has been shown to promote the growth, survival and migration of various cells, including endothelial cells, and has also been shown to be involved in the regulation of epithelial–mesenchymal interactions.[13] In addition, the

* Corresponding author. Tel./fax: +81 95 819 7644.
E-mail address: tohrupth@nagasaki-u.ac.jp (T. Ikeda).

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expression of MK was found to be increased in various human tumors,[13–15] and has also been suggested to promote or modulate angiogenesis.[16–18]

Including the tongue, most of the oral cavity is visible, and it is considered advantageous for early discovery of cancer, but proper medical knowledge and good medical as well as dental care systems are essential. In the hospital, histopathological diagnosis of the lesion is required to determine the course of treatment. Histopathological diagnosis of operation specimens has additionally been desired to provide information to predict prognosis in concert with clinical and imaging data based on tumor-node-metastasis (TNM) classification of tumors. However, further clinically applicable highly efficient prognosticators of OSCC have not been approved, although many possible candidates could be introduced. In this study, an attempt was made to improve one of the reliable histopathological prognosticators for vascular invasion using multiple vasculogenesis-associated factors, VEGF-A, VEGF-C, COX-2 and MK.

Materials and methods

Patients and materials

Ninety specimens of patients with previously untreated OSCC, who underwent either biopsy or surgery with or without preoperative treatment, were included. Patients were admitted to the Second Department of Oral and Maxillofacial Surgery, Nagasaki University Dental Hospital, from 1991 to 2002. They were composed of 58 male and 32 female patients ranging from 31 to 87 years of age with a mean age of 65.4 years. Forty-eight patients were treated with a standard program of preoperative irradiation of Linac at a total of 30 Gy and preoperative continuous subcutaneous administration of peplomycin (5 mg/day; maximum dosage, 100 mg). Nine patients were treated only with the preoperative irradiation, 12 patients were treated only with the preoperative administration of peplomycin and 21 patients were untreated before surgery. In histopathological diagnosis, 72 cases were well-differentiated, 12 cases were moderately differentiated and six cases were poorly differentiated OSCCs. All the patients were followed at the hospital until 2005. Among them, 66 patients (73.3%) died and 24 patients (26.7%) survived during the follow-up period. This study was approved by the ethics committee of the Nagasaki University Graduate School of Biomedical Sciences.

Histopathological analyses

Specimens were routinely processed with a 10% buffered formalin fixative and embedded in paraffin. Morphology of tumor cells was evaluated using specimens stained with hematoxylin and eosin, and the presence of vascular invasion (v-factor) was evaluated using specimens stained with the method of Elastica van Gieson (EVG). Antibodies for vascular endothelial growth factor (VEGF)-A, VEGF-C (both from Zymed Laboratories Invitogen Immunodetection, San Francisco, CA), cyclooxygenase (COX)-2 (Cayman Chemical, Ann Arbor, MI) and Midkine (MK) (Yamasa, Chiba, Japan) were used. Sections were pretreated with an autoclave at 121 °C for 15 min in 10 mM citrate buffer (pH 6.0) to activate antigens, incubated with each antibody described above at ×100 dilution with PBS at 4 °C overnight and immunohistochemical analysis was carried out on the EnVision + System (Dako, Carpinteria, CA).

The expression of VEGF-A, VEGF-C and COX-2 was graded according to the percentage score of the stained carcinoma cells as follows: no expression (code 0), weakly positive expression (code 1) where less than 25% were positive carcinoma cells, moderately positive expression (code 2) where more than 25% to less than 50% were positive carcinoma cells and strongly positive expression (code 3) where more than 50% were positive carcinoma cells under magnifications of 100× and 200×. Owing to the general low intensity of MK immunoreactivity, the expression was ranged from no expression (code 0), weak expression (code 1), which was defined as less than 50% being positive carcinoma cells, to firm expression (code 2), in which more than 50% were positive carcinoma cells. Because VEGF-A, VEGF-C, COX-2 and MK were

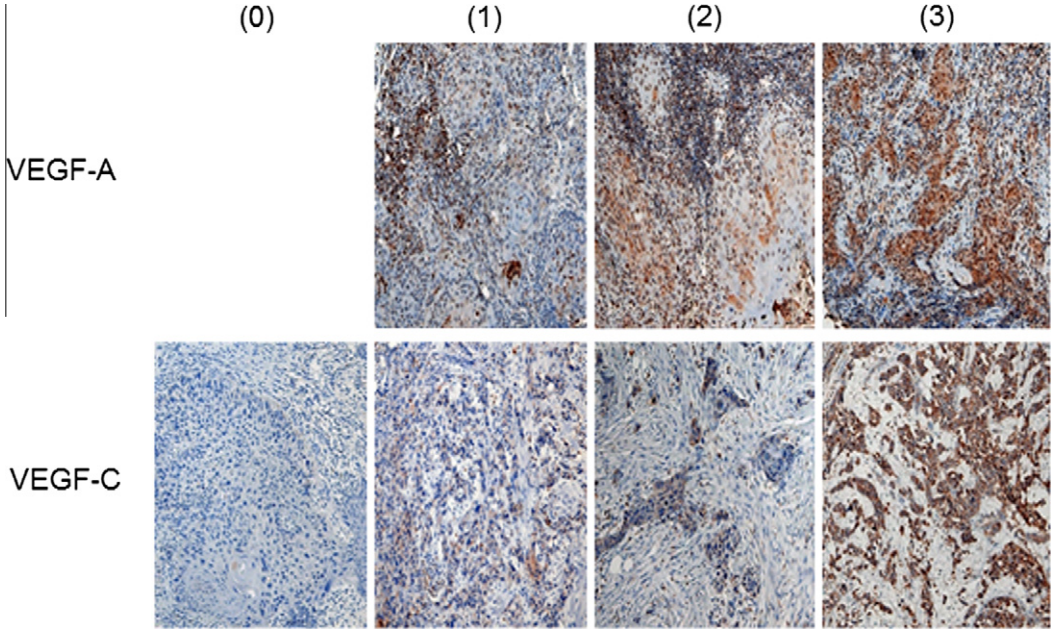

Figure 1 Representative expression profiles of VEGF-A and VEGF-C in immunohistochemical analysis. (0), (1), (2) and (3) represent code 0 (no expression), code 1 (less than 25% of positive carcinoma cells), code 2 (more than 25% to less than 50% of positive carcinoma cells) and code 3 (more than 50% of positive carcinoma cells) of immunohistochemical grading of VEGF-A and VEGF-C. There was no case of code 0 for the expression of VEGF-A.