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ORIGINAL ARTICLE

Upregulation of vascular endothelial growth factor mRNA level is significantly related to progression and prognosis of oral squamous cell carcinomas

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KEYWORDS
cancer progression;
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Background/purpose: Vascular endothelial growth factor (VEGF) is a potent angiogenic factor. This study evaluated whether the VEGF mRNA level in oral squamous cell carcinoma (OSCC) tissue could be a biomarker to predict the progression and prognosis of OSCCs in Taiwan.
Methods: This study used quantitative real-time reverse transcription-polymerase chain reaction (quantitative RT-PCR) to detect the VEGF mRNA levels in 60 OSCC specimens. Threshold cycle (C_T) was defined as the PCR cycle number needed to generate a predetermined amount of DNA (threshold). The relative amount of tissue VEGF mRNA, standardized against the amount of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA, was expressed as ΔC_T = (VEGF C_T − GAPDH C_T). For a chosen threshold, a smaller starting copy number of mRNA results in a higher C_T value. Thus, the lower the ΔC_T, the greater the copy number of VEGF mRNA in tissues.
Results: The lower mean VEGF mRNA ΔC_T value was significantly associated with OSCCs with larger tumor size ($p = 0.040$), positive lymph node metastasis ($p = 0.023$), and more advanced clinical stages ($p = 0.008$). VEGF mRNA ΔC_T value < 4.2 ($p = 0.026$) was identified as an independent unfavorable prognosis factor using multivariate regression analyses. Moreover, Kaplan–Meier curve showed that OSCC patients with a VEGF mRNA ΔC_T value < 4.2 had a significantly poorer overall survival than those with a VEGF mRNA ΔC_T value ≥ 4.2 (log-rank test, $p = 0.0427$).

Conflicts of interest: The authors have no conflicts of interest relevant to this article.

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Conclusion: The OSCC tissue VEGF mRNA level can be used to predict the progression and prognosis of OSCCs in Taiwan.

Introduction

Oral squamous cell carcinoma (OSCC) is the most frequent malignancy of the oral cavity and late-stage OSCCs with neck metastasis often associated with poor prognosis of patients.[1] In Taiwan, the mortality rate of head and neck cancers is in fifth place in the overall population and the fourth place in male populations in 2011.[2]

OSCC is an aggressive epithelial neoplasm. Despite the advances in early detection and treatment of OSCC in recent years,[3] the overall survival rate of OSCC is still not promising, probably due to the lack of a good marker for early diagnosis and prediction of the progression and prognosis of OSCC. Vascular endothelial growth factor (VEGF) can promote angiogenesis which is essential for cancer growth and metastasis.[4–6] Therefore, VEGF protein or mRNA may be a good marker for prediction of cancer progression and prognosis.

VEGF can increase vascular permeability, promote endothelial cell proliferation and migration, and inhibit endothelial cell apoptosis.[5,6] In humans, the gene encoding VEGF is located on the short arm of chromosome 6 (6p21.3).[7] VEGF is a mitogen for vascular endothelial cells and helps the migration and organization of vascular endothelial cells for neovascularization and tumor micrometastasis.[8,9] Overexpression of VEGF mRNA or protein has been associated with aggressive progression and poor prognosis in several human cancers, including colorectal,[10–12] gastric,[13–15] pancreatic,[16,17] and breast carcinomas[18–20] as well as melanoma.[21]

VEGF is also found to be an important angiogenic cytokine for neovascularization in head and neck cancers.[22–29] Overexpression of VEGF mRNA or protein has been reported to be significantly related to poor prognosis and shorter survival[22–27] as well as positive lymph node metastasis[23,28,29] in head and neck cancer patients. In this study, we evaluated whether the VEGF mRNA level of OSCC surgical specimens could be a crucial biomarker to predict the progression, recurrence, and prognosis of OSCCs.

Methods and materials

Patients and oral cancer specimens

Sixty OSCC patients (55 men and 5 women; mean age 56 years; range, 36–81 years) were included in this study. This study has been reviewed and approved by the Institutional Review Board of the National Taiwan University Hospital, Taipei, Taiwan. For each patient and each normal control patient, an informed consent was obtained before collection of surgical samples. Surgical samples of OSCC were collected from 60 OSCC patients. Moreover, 38 biopsy specimens of normal oral mucosa (NOM) were obtained from 38 controls (30 men and 8 women; mean age 33 years; range, 17–55 years) with no oral habits or any oral mucosal diseases during extraction of an impacted permanent lower third molar and these were used as the normal controls. All sample tissues were freshly embedded in optimum cutting temperature compound (Fisher Scientific, Hanover Park, IL, USA), snap frozen, and kept at −80°C until use. VEGF mRNA levels in both OSCC and NOM tissues were measured by quantitative real-time reverse transcription-polymerase chain reaction (quantitative RT-PCR). OSCC was diagnosed with histological examination of hematoxylin and eosin-stained tissue sections.

All OSCC patients underwent total surgical excision of their tumors plus either selective or radical neck dissection based on clinically nodal metastasis at the Department of Oral and Maxillofacial Surgery, National Taiwan University Hospital during the period from January 2002 to December 2009. Follow-up duration was defined as the period between the operation date and day of the last visit, according to the patient's chart. If involved surgical margin, perineural invasion, or lymphovascular permeation of OSCC, or extracapsular spread of metastatic cervical lymph node were detected histologically, concurrent postoperative chemoradiation therapies were also included in the treatment protocol. In this study, 17 patients underwent postoperative chemoradiation therapies due to the presence of aforementioned risk factors of recurrences. However, none of our patients had received any form of tumor-specific therapy or induction chemotherapy before total surgical excision of the lesion. Moreover, there were 33 (55.0%) patients with local recurrences or regional neck metastases and four (6.7%) patients with distant lung metastases. Salvage treatments for patients with local regional recurrences were excision of recurrent local cancers and neck dissection and those for patients with distant lung metastases were palliative chemotherapy. Of the 60 OSCC cases, 22 were buccal mucosa cancers, 17 tongue cancers, and 21 gingiva cancers, palate cancers, or floor of the mouth cancers. Histological features of OSCC were further classified into three different types (well-, moderately-, and poorly-differentiated OSCC). Of the 60 OSCC cases, there were 51 well-differentiated and nine moderately-differentiated OSCCs. Clinical staging and TNM status of OSCCs at initial presentation of the tumor were determined using clinical palpation, head-and-neck magnetic resonance imaging, chest X-ray, abdominal sonography, and whole body bone scan according to the guidelines from the American Joint Committee on Cancer (6th edition).[30]