Vascular Endothelial Growth Factor Receptor Isoforms: Are They Present in Oral Squamous Cell Carcinoma?

Alix Pianka, Thomas Knösel, MD, PhD,† Florian Andreas Probst, MD, DMD,‡ Markus Troeltzsch, MD, DMD,§ Timothy Woodlock, MD,‖ Sven Otto, MD, DMD, PhD,¶ Michael Ehrenfeld, MD, DMD, PhD,# and Matthias Troeltzsch, MD, DMD***

Purpose: Although the clinical importance of vascular endothelial growth factor (VEGF) overexpression in oral squamous cell carcinoma (OSCC) has been investigated, there are limited data about the overexpression of VEGF receptors (VEGF-Rs) and their clinical importance. VEGF-R isoforms have proven influence on proliferation rates, metastasis, and survival in different neoplasms. This study was conducted to investigate VEGF-R expression levels in OSCC samples and to identify any clinical relevance.

Materials and Methods: A retrospective cohort study design (n = 50) was used. Clinical data were gathered from patient charts. Validated immunohistochemical methods were applied to determine VEGF-R isoform expression by tumor cells. Descriptive and inferential statistics with respect to the variable scale were computed. The significance level was set at a *P* value less than or equal to .05.

Results: This study found overexpression of different VEGF-R isoforms in 88% of examined specimens. Statistically important associations were detected between overexpression of specific VEGF-Rs and tumor size, neck node metastasis, and tumor-associated death. Furthermore, a history of common OSCC risk factors (smoking and alcohol consumption) were found considerably more often in patients whose OSCC specimens displayed VEGF-R overexpression.

Conclusion: These findings show that VEGF-R overexpression occurs frequently in OSCC and could have clinical implications.

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Oral squamous cell carcinoma (OSCC) is the sixth most common malignancy worldwide. It is characterized by an aggressive behavior owing to frequent and early lymph node metastasis and early recurrence.[1] Although some progress in surgical therapy, radiation, and chemotherapy has occurred, no meaningful benefit in long-term prognosis has been achieved.[2] Although the classic risk factors of smoking and alcohol consumption contribute considerably to OSCC pathogenesis,[3] the importance of other potential causalities,

*Graduate Student, Department of Oral and Maxillofacial Surgery, Ludwig Maximilians University of Munich, Munich, Germany.

†Associate Professor, Department of Pathology, Ludwig Maximilians University of Munich, Munich, Germany.

‡Resident, Department of Oral and Maxillofacial Surgery, Ludwig Maximilians University of Munich, Munich, Germany.

§Resident, Department of Oral and Maxillofacial Surgery, Georg - August University of Göttingen, Germany.

‖Medical Oncologist, Division of Medical Oncology and Hematology, Department of Medicine, School of Medicine and Dentistry, University of Rochester; and Unity Health System, Rochester, NY.

¶Associate Professor, Department of Oral and Maxillofacial Surgery, Ludwig Maximilians University of Munich, Munich, Germany.

#Professor and Department Chair, Department of Oral and Maxillofacial Surgery, Ludwig Maximilians University of Munich, Munich, Germany.

**Resident, Department of Oral and Maxillofacial Surgery, Ludwig Maximilians University of Munich, Munich, Germany.

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Address correspondence and reprint requests to Dr Troeltzsch: Department of Oral and Maxillofacial Surgery, Ludwig Maximilians University of Munich, Lindwurmstraße 2a, 80337 Munich, Germany; e-mail: matthias_troeltzsch@hotmail.com

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such as infection with human papilloma virus (HPV), remains under scientific debate.[4,5]

Improved understanding of oropharyngeal SCC carcinogenesis has enabled the introduction of clinically successful targeted therapies, including cetuximab, an antibody against epithelial growth factor receptor expressed in OSCC, with beneficial effects on recurrence rates and midterm survival.[6] Efforts are underway to identify other biologic targets within oral and oropharyngeal SCC, with potential clinical implications.[7-9]

Vascular endothelial growth factors (VEGFs) and their receptors (VEGF-Rs) have attracted attention because of their importance in the pathogenesis of colon, ovarian, lung, and other cancers.[9,10] Moreover, anti-VEGF agents, such as the monoclonal antibody bevacizumab, have shown marked patient benefit in clinical trials and are currently available commercially for cancer treatment.[11] VEGF-R1 and 2 mediate mitogenic functions and induce angiogenesis, among other functions,[12] whereas VEGF-R3 plays a critical role in lymphangiogenesis.[13] As tyrosine kinases, VEGF-Rs can be the aim of targeted therapies.[14] Although the influence of VEGF and its isoforms in head and neck SCC (HNSCC) is well researched,[11,15] there are only limited and controversial data about the implications of VEGF-R and its isoforms (VEGF-R1, 2, and 3) on clinical parameters of HNSCC or OSCC alone.[15,16] There is evidence that OSCC cells can express VEGF isoforms and their receptors at the same time, highlighting an autocrine and a paracrine function of these biomarkers.[12,13,15-18] Studies have found associations between HPV infection of HNSCC and VEGF overexpression,[19] whereas no data on this topic are available for VEGF-Rs.[20] If a link does exist among HPV, VEGF, and VEGF-R, the impact of HPV infection could be biologically important by enabling an autocrine growth stimulatory pathway. Although data are limited, various investigators have suggested an association between the presence of VEGF-R3 in OSCC tumor cells and the presence of lymphatic metastases and prognosis.[13,21] These novel concepts require further study, and indeed the presence and magnitude of expression of the other VEGF-R isoforms remain poorly defined[15,22,23] and open to further investigation.

The aim of the present study was to analyze a randomly chosen predefined cohort of strictly OSCC specimens for the overexpression of VEGF-R isoforms and to identify possible associations of noteworthy VEGF-R presence on OSCC specimens with clinical and pathologic parameters. Furthermore, the question of possible associations between HPV infection of OSCC cells and VEGF-R overexpression should be clarified. The authors hypothesized that there are no clinical implications of VEGF-R overexpression in OSCC and that VEGF-R overexpression is not associated with HPV infection.

Materials and Methods

STUDY DESIGN

A retrospective cohort study was performed. The random number generation function of Excel (2011; Microsoft, Redmond, WA) was used to select 50 patients as a representative sample of the entire patient population treated surgically for primary manifestation of OSCC at the Department of Oral and Maxillofacial Surgery, Ludwig Maximilians University of Munich, Germany from 2006 through 2012.[4] The exclusion criteria were defined as prior radiotherapy, prior chemotherapy, synchronous HNSCC at other locations, and prior therapy with antiangiogenic drugs. Some of the chosen patients had already been considered for a previous study.[4] The conduct of the study was in concordance with ethical requirements and internal review board approval was obtained. The study design, data acquisition, and article preparation followed previously validated standards.[24,25]

STUDY VARIABLES

The data collection was achieved by reviewing patient records, pathology reports, and immunohistochemistry results. After a thorough literature review,[7-10,16,17,26-28] the following variables were considered appropriate to answer the research question: patient gender, age at first OSCC manifestation, exposure to classic risk factors, such as smoking tobacco (>10 pack-years) and regular alcohol consumption for at least 10 years (smoking and alcohol consumption continued or ceased <10 years before OSCC occurrence),[29] infection with HPV assessed by p16 overexpression,[30] expression of VEGF-R1, 2, and 3, tumor size, neck node involvement, histologic grade, tumor stage at time of primary surgery (as defined by the Union for International Cancer Control [UICC]),[31] follow-up interval, and survival. The variable expression of VEGF-R was defined as the predictor variable and the primary outcome variable was UICC stage. The variables patient gender, exposure to classic risk factors, infection with HPV, expression of VEGF-R, neck node involvement, histologic grade, and survival were coded on an ordinal scale. A nominal scale was used for the classification of the variables tumor size, UICC stage, and histologic grade. An interval scale was used to organize the variables age and follow-up period.

IMMUNOHISTOCHEMISTRY

For tissue microarray (TMA) construction, punch biopsy specimens 0.6 mm in diameter were obtained from representative areas of the OSCC specimens as described elsewhere.[27,28,32] Three biopsy specimens were gathered from each OSCC specimen. The