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Vascularity and expression of vascular endothelial growth factor in oral squamous cell carcinoma, resection margins, and nodal metastases

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Abstract

The role of vascularity as a predictor of the likelihood of lymph node metastases in oral cancer is not clear. To that end, the vascularity and expression of vascular endothelial growth factor (VEGF) was assessed at three specific regions: the tumour (inside and around the tumour); the resection margin; and the regional lymph nodes. Formalin-fixed paraffin-embedded specimens from 26 oral cancers (11 with no involved nodes and 15 with involved nodes) were stained immunohistochemically and examined. Staining for VEFG was significantly greater in the tumour than in the other sites. No significant differences were found in the intensity of staining in the primary tumour, resection margins, or nodes between cases in which the nodes were involved and in which they were not involved. We found no correlation between vascularity and VEGF staining, suggesting that VEGF is not the primary or only stimulator of angiogenesis in oral cancer. Greater understanding of the mechanisms of metastasis will lead to new treatments. The evidence that is accumulating for oral cancer suggests that such treatments may be better targeted at preventing lymphatic spread, rather than vascular spread.

Keywords: VEGF; Oral cancer; Prognostic markers; Metastasis

Introduction

In the United Kingdom squamous cell carcinoma of the head and neck accounts for 900 deaths each year with a death:registration ratio of 1:0.45. Unlike squamous cell carcinomas at other sites, haematogenous metastases are rare. However, spread to regional lymph nodes is common, and nodal metastases are the most important prognostic factor.[1] The growth of solid tumours depends on the formation of new blood vessels (angiogenesis). Angiogenesis is also associated with an increased risk of metastases.[2] However, there are conflicting reports about the prognostic significance of vascularity which is used as an index of angiogenesis. This may be explained by the fact that the normal oral mucosa is richly vascular and may be affected by regional variation so that angiogenesis may be of limited influence.[3,4] Variations in methods compound the interpretation and comparison of studies.[5]

Angiogenesis is regulated by a complex interaction between stimulators and inhibitors. Vascular endothelial growth factor (VEGF) is an endothelial-specific mitogen that plays a pivotal part in angiogenesis.[6] Cell studies have shown that under hypoxic conditions oral tumour cells upregulate the expression of VEGF.[7] Ex-vivo work on oral carcinoma suggests that a high level of expression of VEGF is associated with a poor prognosis[8] and nodal involvement.[9] There is a report of significantly more VEGF stained in carcinomas than in normal oral mucosa,[10] but another study found no difference.[11]

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The aim of our study was to investigate the correlation between vascularity and VEGF in oral squamous cell carcinoma, the resection margins, and nodal metastases to find out whether they are of prognostic importance.

Materials and methods

Records of 26 surgically treated cases of oral squamous cell carcinoma were identified from the archives of the Oral Pathology Department, the University of Birmingham Dental Hospital and School. They came from 13 women and 12 men (the sex of one patient was not recorded) ranging in age from 32 to 83 years with a mean age of 61. Of these 26 cases 11 had no nodal metastases and 15 had nodal metastases. These patients were followed up for up to 51 months and had a mean survival time of 20 months (range 6–51) and a mortality of 38% in that period. Clinical details including use of alcohol and tobacco and survival are shown in Table 1. From each case three tissue specimens were studied consisting of the primary tumour, normal tissue from the periphery of the resection margin, and one associated regional lymph node. In 13 of these cases there was epithelial dysplasia at the periphery of the primary tumour.

Formalin-fixed paraffin-embedded sections (6 μm) were cut from the various sites outlined. Sections were dewaxed in xylene, rehydrated in ethanol and washed in phosphate buffered saline (PBS) for 10 min. Sections were then immersed in 3% hydrogen peroxide in PBS for 30 min before pretreatment with 0.1% protease XXIV (Sigma, Poole, England) for the polyclonal antibody to von Willebrand factor (Dako, UK) or immersed in buffered citric acid (pH 6.2) and microwaved for 10 min for the polyclonal antibody to VEGF (A-20 Santa Cruz Biotechnology, Inc.). Following a wash in PBS the sections were incubated overnight at 4 °C with the primary antibody at a 1:4000 dilution. The sections were then washed in PBS and incubated for 30 min at room temperature with a biotinylated secondary antibody (Vector Laboratories Ltd., Peterborough, England) followed by the Elite avidin biotin complex amplification kit (Vector) and the substrate was made visible by incubation with diaminobenzidine substrate (Sigma). The sections were then counterstained with haematoxylin and dehydrated in graded alcohols, cleared in xylene, and mounted.

Quantification of microvessels and staining for VEGF

The stereological method of point counting was used to quantify the microvasculature to provide an index of angiogenesis. This involved scanning 10 fields across each specimen at

Table 1
Clinical details of the patients

Case	Sex	Age	Site	Status	T	A	Diff.	TNM_O	TR	TD
1	F	60	FOM	Alive	Y	Y	Mod	T_1N_0	–	–
2	M	61	Ton	Dead	Y	Y	Mod	T_2N_0	–	7
3	M	32	BM	Dead	Y	Y	Mod	T_4N_0	–	6
4	–	–	Ton	Alive	N	N	Mod	T_2N_0	–	–
5	M	73	Alv	Alive	Y	Y	Mod	T_4N_0	–	–
6	M	–	FOM	Alive	Y	Y	Mod	T_4N_0	2	–
7	F	63	Ton	Alive	N	Y	Mod	T_2N_0	–	–
8	M	31	BM	Dead	Y	Y	Mod	T_1N_0	6	10
9	F	63	RMP	Alive	Y	Y	Poor	T_3N_0	–	–
10	F	76	Pal	Alive	Y	Y	Mod	T_1N_0	–	–
11	M	49	FOM	Alive	Y	Y	Mod	T_4N_0	–	–
12	M	69	Ton	Alive	Y	Y	Mod	T_2N_2	–	–
13	M	58	Alv	Dead	Y	Y	Mod	T_4N_0	8	9
14	F	72	Ton	Dead	Y	Y	Mod	T_3N_0	–	19
15	F	72	BM	Alive	N	Y	Mod	T_4N_2	–	–
16	F	68	FOM	Dead	Y	Y	Mod	T_4N_3	–	6
17	M	59	Alv	Dead	Y	Y	Mod	T_4N_1	6	38
18	F	68	BM	Dead	Y	Y	Mod	T_4N_1	–	9
19	F	43	Alv	Alive	Y	Y	Mod	T_3N_1	–	–
20	M	72	FOM	Alive	Y	N	Mod	T_2N_1	16	–
21	F	83	BM	Alive	N	N	Mod	T_4N_2	9	–
22	M	51	Ton	Alive	Y	Y	Mod	T_3N_1	–	–
23	M	45	RMP	Alive	Y	Y	Well	T_4N_1	–	–
24	F	60	FOM	Alive	Y	Y	Well	T_2N_1	8	–
25	F	74	RMP	Dead	N	N	Mod	T_4N_2	—	18
26	F	57	Ton	Dead	N	N	Mod	T_3N_0	6	7

The table gives the sex, age, site affected, aetiological factors, histological differentiation, nodal involvement, recurrence rate, and survival status of the 26 patients with oral squamous cell carcinoma. Site: Ton: tongue; FOM: floor of mouth; BM: buccal mucosa; RMP: retromolar pad; Pal: palate; Alv: mandibular alveolus; T: tobacco use; A: alcohol consumption; Y: yes; N: no; Diff.: histological differentiation: well, mod: moderately poor; TR: time to recurrence in months; TD: survival time in months.