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Predictive factor for photodynamic therapy effects on oral squamous cell carcinoma and oral epithelial dysplasia

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ABSTRACT

Objective: The aim of this study was to investigate the correlation between the immunohistochemical expression of proliferating cell nuclear antigen (PCNA), factor VIII, and CD34 (markers of endothelial cells), and vascular endothelial growth factor (VEGF) and the recurrence of oral squamous cell carcinoma (OSCC) and oral epithelial dysplasia (OED) subjected to photodynamic therapy (PDT).

Design: Twenty-one biopsy specimens (14 cases of OSCC and 7 cases of OED) before PDT were immunohistochemically investigated in terms of their expressions of PCNA, factor VIII, CD34 and VEGF. The percentages of the total sample area that were immunopositive for factor VIII (percentage factor VIII immunopositive area: PFIA) CD34 (PCIA) and VEGF (PVIA) were calculated using computer-assisted image analysis for quantitative assessment of endothelial cells or VEGF expression in the lesions. The PCNA labelling index (LI) was evaluated as a proliferation marker.

Results: Five cases of OSCC and one case of OED recurred 4 to 30 months after PDT. We found that the average PVIA was 14.5% in the no-recurrence group and 1.7% in the recurrence group. The difference between these values was statistically significant ($P = 0.0483$). On the other hand, the average PCNA LI was 30.3% in the no-recurrence group and 24.3% in the recurrence group; the average PFIA was 3.7% in the no-recurrence group and 1.6% in the recurrence group; and the average PCIA was 2.0% in the no-recurrence group and 1.4% in the recurrence group. There were no significant differences between the two groups for any of these markers ($P = 0.3379$, $P = 0.1195$, $P = 0.4835$, respectively).

Conclusions: These results provide clinical data indicating that VEGF expression may be a useful predictive marker for the effects of PDT in OSCC and OED.

1. Introduction

Photodynamic therapy (PDT), which consists of administration of a photosensitiser and subsequent laser irradiation, is used for treating a variety of malignant tumours, including oral cancers.[1] However, recurrence of the tumour after PDT has been problematic.[2–4] Because the cytotoxic effects of PDT appear to arise from the generation of singlet oxygen and free radicals due to photodynamic reaction,[5,6] tumour oxygenation should be an important factor for the anti-tumour effects of PDT[7]. In turn, tumour oxygenation is likely to be relevant to the tumour vasculature,[8] and the PDT effects, which lead to rapid tumour necrosis, are likely to be caused by damage to the

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Table 1 – Summary of patients.

Case (age, sex)	Site of lesion(s)	Histological diagnosis	Follow up or recurrent term	Recurrence
1 (56, F)	Tongue (T2N0M0)	SCC, well differentiated	30 months	+
2 (90, F)	Buccal mucosa (T2N0M0)	SCC, well differentiated	15 months	+
3 (69, F)	(i) lt. buccal mucosa (T1N0M0)	SCC, well differentiated	11 months	+
	(ii) rt. mandibular gingiva (T1N0M0)	SCC, well differentiated	37 months	–
	(iii) rt. buccal mucosa (T1N0M0)	SCC, well differentiated	37 months	–
4(83, M)	Hard palate (T2N0M0)	SCC, well differentiated	15 months	+
5(70, M)	Oral floor (T2N0M0)	SCC, well differentiated	4 months	+
6(63, F)	Tongue (T2N0M0)	SCC, well differentiated	44 months	–
7(80, F)	Hard palate (T1N0M0)	SCC, well differentiated	24 months	–
8(85, F)	Maxillary gingiva (T2N0M0)	SCC, well differentiated	22 months	–
9(75, M)	Buccal mucosa (T1N0M0)	SCC, well differentiated	20 months	–
10(74, F)	Mandibular gingiva (T1N0M0)	SCC, well differentiated	15 months	–
11(78, F)	Mandibular gingiva (T1N0M0)	SCC, well differentiated	18 months	–
12(76, M)	Tongue (T2N0M0)	SCC, well differentiated	18 months	–
13(72, M)	Tongue	ED, moderate	19 months	–
14(29, F)	Tongue	ED, mild	19 months	–
15(79, F)	Hard palate	ED, mild	30 months	–
16(65, F)	Tongue	ED, severe	30 months	+
17(61, M)	Soft palate	ED, mild	33 months	–
18(70, F)	Buccal mucosa	ED moderate	16 months	–
19 (67, M)	Tongue	ED, mild	7 months	–

Abbreviations: SCC, squamous cell carcinoma; ED, epithelial dysplasia.

endothelial cells of the tumour.[9,10] Vascular endothelial growth factor (VEGF) is a heparin-binding, dimeric polypeptide growth factor with mitogenic activity specific for vascular endothelial cells.[11,12] Hypoxic or anoxic conditions induce VEGF secretion in tissue,[13] and this cytokine is likely to be a prognostic marker for oral squamous cell carcinoma (OSCC).[14] Accordingly, it seemed worthwhile to investigate the correlation between endothelial cells as well as the expression of VEGF in PDT target lesions and the clinical results of PDT.

On the other hand, the decrease of proliferating cell nuclear antigen labelling indices (PCNA LIs) of tumour cells after PDT is likely to reflect the PDT anti-tumour effects,[15] just as tumour PCNA LIs appear to be useful prognostic markers for oral cancer.[16,17] Accordingly, it would also be worthwhile to estimate the correlation between PCNA LIs of PDT target lesions and the clinical results of PDT.

We present here 14 cases of OSCC and 7 cases of oral epithelial dysplasia (OED) treated by PDT (Table 1). None of the biopsies taken at 4 weeks after PDT showed evidence of residual tumour. However, 5 cases of OSCC and one case of OED recurred 4–30 months after PDT. The biopsy specimens before PDT were immunohistochemically investigated in terms of the expression of factor VIII and CD34 (markers of endothelial cells), PCNA, and VEGF. Furthermore, quantification of VEGF, factor VIII and CD34 expression was carried out using computer-assisted image analysis to compare the difference between cases in the recurrence and no-recurrence groups.

2. Material and methods

2.1. *Patients and PDT*

Twenty-one lesions with a histologically proven squamous cell carcinoma (Fig. 1) or epithelial dysplasia of the oral cavity were included. Mild epithelial dysplasia was characterised by the hyperchromatic and slightly pleomorphic nuclei in the basal and parabasal cell layers of the stratified squamous epithelium. Dysplastic changes extended to the midpoint of the epithelium and were characterised by nuclear hyperchromatism, pleomorphism, and cellular crowding in moderate epithelial dysplasia. In the cases of severe dysplasia, dysplastic epithelial cells extended from the basal layer to the surface of the mucosa without invasion, and there was abnormal proliferation.[18] All lesions were diagnosed as stage I or II (T1 or 2 N0M0) OSCC or OED, and no deep extension to underlying muscle or bone was suggested by the clinical, histological, computed tomography (CT) or magnetic resonance imaging (MRI) findings. There was no case of carcinoma *in situ* in the present series. None of the patients had received prior treatment. Informed consent was obtained in each case.

Photofrin® (sodium porphymer; Wyeth Lederle Japan, Tokyo, Japan) at a dose of 2 mg/kg was given by intravenous infusion 48 h prior to surface laser irradiation. An excimer dye laser (PDT EDL-1; Hamamatsu, Hamamatsu-shi, Japan) was used to deliver monochromatic red light at 630 nm. An optical fibre was applied to irradiate the targeted oral lesions. A light dose of 100–200 J/cm^2 was delivered to each area. The patients were counselled to avoid direct sunlight for six weeks following sensitisation. Treated areas were biopsied to confirm the effects of PDT 4 weeks after laser irradiation.

A complete response was defined as the disappearance of all known disease at 4 weeks after PDT. The patients were followed every 3 months using cytology as well as clinical findings. Surgical resection was carried out as a post-recurrence treatment to provide additional protection.

2.2. *Immunohistochemistry of PCNA, VEGF, factor VIII and CD34*

Four sets of 4-μm sections were prepared for each specimen, mounted on poly L-lysine-coated glass slides, and dried