Immunolocalization of Endothelial Markers in Oral Squamous Cell Carcinoma and Their Relationship with Metastasis

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Abstract: Intratumoral blood vessels are known to play an important role in cancer growth and metastasis. The discrepancy in previous reports using various endothelial markers individually suggested us to investigate both normal and various tumor areas with a wide panel of vascular markers. We used a panel of three antibodies (CD31, CD34 and endoglin) as blood vessel markers to investigate the distribution and properties of blood vessels in normal oral tissues and squamous cell carcinomas. Many microvessels with strong remodeling activity as well as undifferentiated tumoral vascular endothelial cells and immature endothelial cells were present in the cancer cell nest and marginal area of cancer infiltration. Our results showed different vascular distribution patterns using various immunostaining markers in normal and tumoral tissues. Vascular distribution and properties of endothelial cells appear to be closely associated with metastasis.

Key Words: squamous cell carcinoma, oral cancer, endothelial marker, metastasis

Introduction

Intratumoral blood vessels are known to play an important role in cancer growth by supplying oxygen and nutrients as well as excreting metabolic products, and to be associated with metastasis. Previous reports on the identification of tumoral blood vessels generally employed anti-factor VIII antibody, anti-CD31 antibody (CD31), or anti-CD34 antibody (CD34) individually as vascular marker. Recent studies on vascular endothelial cells have identified endoglin, which is a homodimeric glycoprotein of endothelial cells. Endoglin has been shown to have high binding affinity to TGF-b1 and –b3. Anti-CD105 antibody (CD105) is the antibody against endoglin, and has been reported to be useful in identifying specifically neovessels of tumors.

The purpose of this study was to use multiple antibodies, including new anti-CD105 antibody and other vascular markers, to investigate the distribution of microvessels and the properties of vascular endothelial cells in oral squamous cell carcinoma (OSCC), and to examine the correlation with lymph node metastasis.

Materials and methods

Tissue samples

Samples stored at the department of oral pathology Okayama University hospital were used in the study. Forty cases of OSCC were studied, including 18 cases with lymph node metastasis. For the degree of differentiation in 18 metastatic tumors, 8 cases were well differentiated, 3 were moderately differentiated, and 7 were poorly differentiated. For the 22 cases without metastasis were as follows: 14 cases were well differentiated, 4 were moderately differentiated, and 4 were poorly differentiated. Five samples of normal mucosal tissue were used as control.

All the tissue samples were fixed in 10% neutral buffered formalin and paraffin-embedded according to the conventional methods. From each tissue block, 4 µm serial sections were prepared.

Immunohistochemical staining

Vascular endothelial cell markers were used as the first antibodies, comprising anti-CD31 antibody (JC/70A, Dako Corp.), anti-CD34 antibody (Nu-44A1, Nichirei Corp.), anti-CD105 antibody (Sn6h, Dako Corp.). To identify vascular basement membrane, anti-type IV collagen antibody (H11, Shigei Medical Lab. Inst., Okayama, Japan) was used as the first antibody. After deparaffinization, the serial sections were immersed in 0.03% hydrogen peroxide in methanol. Immunohistochemical staining was performed by the ABC method (Vector Laboratories). Color was developed with 3,3’-diaminobenzidine tetrahydrochloride. The sections were counterstained with Mayer’s hematoxylin and examined under a light microscope.

Results and Discussion

Immunostaining pattern in oral squamous cell carcinoma

Cancer nest area: Anti-type IV collagen antibody immunohistochemically stained the type IV collagen in the vascular basement membrane. Two patterns of vascular distribution were observed in the cancer nest area. For the first pattern, microvessels encircled the cancer cell nest but did not invade the cancer nest. This pattern was designated as circumscribing type (Fig. 1a). For the second pattern, the stroma was narrow, and microvessels invaded the cancer cell nest. This type was designated as penetrating type (Fig. 1b).

Two immunostaining patterns of the vascular endothelial cells
were observed in the cancer nest area. The first pattern was type A (positive for CD31, CD34 and CD105. Type A blood vessels appeared to encircle individual cancer cell nests, and were commonly observed in the circumscribing type tumors. The second pattern was CD31-negative, CD34-positive, and CD105-positive. This pattern was classified as type C. Type C blood vessels were commonly found in the penetrating type tumors.

**Cancer infiltration marginal area:** The immunostaining pattern of vascular endothelial cells in the marginal area of cancer infiltration was type C (CD31-negative, CD34-positive, and CD105-positive). These endothelial cells showed irregular orientation and did not form a definite lumen.

**Cancer infiltration peripheral area:** In the areas peripheral to cancer infiltration, blood vessels were dilated irregularly. The number of cuboidal endothelial cells with enlarged nucleus increased, and aligned along the tubular lumen wall. These blood vessels were all type A (CD31-positive, CD34-positive and CD105-positive) (Fig. 2a,b).

**Vascular distribution and lymph node metastasis:** We used chi-squared test to analyze the correlation between lymph node metastasis and the two types of vascular distribution (circumscribing type with type A pattern and penetrating type with predominantly type C pattern) in cancer cell nest area. Lymph node metastasis was significantly more frequent in penetrating type cases than in circumscribing type cases. 6 of 21 (28%) cancer nest with circumscribing type vessel showed lymph node metastasis while 12 of 19 (63%) of cancer nest with penetrating type vessels were metastatic.

Previous study on vascular distribution in squamous cell carcinomas only reported a high vessel count in the tissues surrounding foci of cancer invasion. There is so far no report that investigates the vascular distribution and properties in defined regions of the cancer lesion; namely, cancer nest area, cancer infiltration marginal area and cancer infiltration peripheral area. The results of the present study clearly demonstrated different immunostaining patterns of the endothelial cells in the three regions, suggesting that there are differences in distribution of vessels and properties of endothelial cells in these regions. Therefore, accompanying proliferation of cancer cells, the tumoral blood vessels in the cancer cell nests and marginal area of cancer infiltration are constantly extending endothelial cells and actively undergoing remodeling.

The present study revealed the presence of a large number of immature vascular endothelial cells in the cancer cell nest and margin of cancer infiltration, and the blood vessels in the cancer nest could be divided into the circumscribing and penetrating types. Furthermore, this study also demonstrated that cases of the penetrating type containing mostly immature blood vessels had a higher frequency of lymph node metastasis.

The finding that the penetrating type correlates with metastasis suggests that metastasis is closely associated with the distribution of blood vessels in the cancer cell nest and marginal area of cancer infiltration, together with the immature property of endothelial cells.

**References**


![Fig. 1 Immunohistochemical staining by anti-type IV collagen antibody shows positive staining along the lumen of blood vessel encircling the cancer nest (1a, circumscribing type) and in lumens of blood vessels inside the cancer cell nest and surrounding the cancer nest (1b, penetrating type).](image1)

![Fig. 2 Positive immunohistochemical stainings of serial sections by anti-CD31 antibody (2a) and by anti-CD105 antibody (2b) are clearly observed in vascular endothelial cells.](image2)