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Significance of the expression of integrin β1, VEGF and MVD in hypopharyngeal squamous cell carcinoma

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ABSTRACT. This study aimed to determine the expression of integrin β1 and vascular endothelial growth factor (VEGF) and microvascular density (MVD) by CD105 staining in hypopharyngeal squamous cell carcinoma to determine their association with clinicopathologic characteristics, and to determine their role and the effects of their interactions in the development and progression of hypopharyngeal squamous cell carcinomas. The expression of integrin β1 and VEGF and MVD in hypopharyngeal squamous cell carcinomas and normal hypopharyngeal tissues were evaluated using immunohistochemistry. The Image-Pro Plus software was used to determine the mean optical density of the immunohistochemical images. Integrin β1 expression was significantly higher in hypopharyngeal squamous cell carcinoma tissues (78.00%) than in normal hypopharyngeal tissues (35.00%; P = 0.001) and significantly differed across pathologic grades and different T stages, and regarding the presence of cervical lymph node metastasis (P < 0.05). VEGF expression was significantly

higher in hypopharyngeal squamous cell carcinoma tissues (74.00%) than in normal hypopharyngeal tissues (30.00%; P = 0.002), VEGF overexpression differed significantly across different pathologic grades and different T stages, and regarding the presence of cervical lymph node metastasis (P < 0.05). The MVD count was significantly higher in hypopharyngeal squamous cell carcinoma tissues (37.10 ± 5.95) than in normal hypopharyngeal tissues (8.70 ± 3.34; P = 0.000). MVD differed significantly across different pathologic grades and different T stages, and regarding the presence of cervical lymph node metastasis (P < 0.05). The expression of integrin β1 and VEGF and the MVD count exhibited no significant differences in terms of age, gender, history of smoking, and clinical stages (P > 0.05). VEGF expression was positively associated with the MVD count of hypopharyngeal squamous cell carcinomas (r = 0.582, P = 0.000); however, integrin β1 was not associated with VEGF or MVD (P > 0.05). Integrin β1 and VEGF are overexpressed and MVD increased in hypopharyngeal squamous cell carcinomas. VEGF is positively correlated with MVD.

Key words: Integrin β1; Vascular endothelial growth factor; Microvascular density; Hypopharyngeal neoplasms; Squamous cell carcinoma; Immunohistochemistry

INTRODUCTION

Hypopharyngeal carcinoma is a primary malignant tumor of the hypopharynx, accounting for 1.4 to 5.0% of all head and neck cancers and 0.5% of systemic malignancies. Up to 95% of hypopharyngeal neoplasms are squamous cell carcinomas, which are characterized by poor cellular differentiation and concealed positions. The early stages of hypopharyngeal carcinoma have no specific symptoms, with all clinical symptoms appearing relatively late. Hypopharyngeal squamous cell carcinoma is characterized by local invasive growth and spreading along the submucosa. Hypopharyngeal squamous cell carcinoma often shows lymph node metastasis and distant metastasis, with 50% of cases having cervical lymph node metastasis at the time of cancer diagnosis (Buckley and MacLennan, 2000). Consequently, it is clinically difficult to diagnose and has a poor prognosis. Therefore, to improve patient survival, we need to find related indicators and evaluate the biological behavior of malignant tumors using an accurate and easy diagnostic method to help providing timely and reasonable comprehensive treatments.

Malignant tumor growth and metastasis depend on angiogenesis. In the early 1970s, Folkman and Shing (1992) first proposed that tumor growth and metastasis depend on the formation of new blood vessels. Since then, angiogenesis and cell adhesion have proven to be closely related to tumor occurrence, development, and metastasis. Consequently, finding an effective method for preventing tumor angiogenesis has become a research focus for current cancer treatments. Thus, the present study focused on the relationship of cell adhesion molecules, cytokines, and hypopharyngeal cancer angiogenesis with tumor growth. We searched for prognostic indicators for hypopharyngeal cancer. These observations provide a reasonable theoretical basis for the early diagnosis of hypopharyngeal cancer, as well as a guide for comprehensive treatment.

The study finds that cell adhesion has an important role in tumor growth and invasion. Moreover, angiogenesis is the key link in tumor growth. Integrin β1 is a transmembrane glycoprotein that mediates the interplay between cells and the extracellular matrix (ECM), i.e., cellular interactions. Integrin β1 participates in cell migration, differentiation, proliferation, and apoptosis (Ohene-Abuakwa and Pignatelli, 2000). Integrin functions in 1) intercellular adhesion, i.e., the connections between cells and the ECM, 2) cell information transfer inside and outside, and 3) mediating angiogenesis. Vascular endothelial growth factors (VEGF) increase vascular permeability, causing swelling of the ECM, and promote the formation of new vessels and cell differentiation through a large quantity of fibrin. Differentiation of microvascular density (MVD) is the final effector of angiogenesis, and is used as a marker for the degree of tumor angiogenesis. We used the PV-9000 two-step immunohistochemical method and analyzed the differential expression of integrin β1, VEGF and MVD using CD105 staining between hypopharyngeal squamous carcinoma tissues and normal hypopharyngeal tissues to explore the association of these factors with the clinicopathologic characteristics and their role in the development and progression of hypopharyngeal squamous cell carcinoma.

MATERIAL AND METHODS

Specimens

Surgical specimens were collected from 50 cases of hypopharyngeal squamous cell carcinoma (3 females and 47 males). The inclusion criteria were as follows: primary hypopharyngeal squamous cell carcinoma confirmed through histopathology, well-preserved specimens, complete clinical records and pathologic data, and no anti-tumor treatment before operation, including radiotherapy, chemotherapy, biotherapy, and so on. The specimens were collected from January 1996 to August 2010. The age of the subjects ranged from 42 to 79 years, with an average of 56.8 years. The specimens were classified according to the location of the primary tumor: 26 were in the pyriform fossa, 15 were in the posterior pharyngeal wall, and 9 were in the postcricoid area. Clinical staging, based on the criteria by the International Union against Cancer, was as follows: T_1 to T_2, 20 cases (4 cases of T_1, 16 cases of T_2); T_3 to T_4, 30 cases (22 cases of T_3, 8 cases of T_4). The specimens were also classified in terms of degree of differentiation: 9 cases were poorly differentiated carcinomas, 26 were moderately differentiated carcinomas, and 15 were well-differentiated carcinomas. Of the 50 cases, 43 were positive for cervical lymph node metastasis and 7 were negative. Up to 20 specimens of normal hypopharyngeal mucosa from paraneoplastic tissue of hypopharyngeal neoplasms, the postcricoid area, and pyriform fossa after total laryngectomy were used as the control. The specimens were cut without the aid of microscopy and were confirmed to be free of cancer cells under hematoxylin-eosin staining. This study was conducted in accordance with the Declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Fujian Medical University. Written informed consent was obtained from all participants.

Experimental methods

The PV-9000 two-step immunohistochemical method was strictly followed (immunohistochemistry assay kit of PV-9000 two-step method). The specimens were dewaxed and endogenous peroxidase activity was blocked. The antigens were retrieved using EDTA antigen

repair buffer. The specimens were labeled with the primary antibodies [rabbit anti-human integrin β1 polyclonal antibodies (SC-8978), rabbit anti-human VEGF monoclonal antibodies (ZA-0509), mouse anti-human CD105 monoclonal antibodies (ZM-0297, ready to use), and antibody diluent (ZLI-9029)]. The sections were then washed with phosphate-buffered saline (PBS) under sonication, stained with diaminobenzidine (DAB) using a DAB color development kit (Type ZLI-9018), counterstained with hematoxylin, and sealed with neutral gum. The rabbit anti-human integrin β1 polyclonal antibodies were purchased from Santa Cruz Biotechnology, Inc. (Dallas, TX, USA). The other antibodies were purchased from the ZSGB-BIO Company of Beijing, Inc. (Xuanwu District, Beijing, China). We used integrin β1-positive breast cancer tissue and VEGF- and MVD-CD105-positive angiosarcoma tissue as the positive controls. The primary antibodies were replaced with PBS in the negative control.

Assessment and analysis

Positive integrin β1 expression was indicated by brownish yellow particles in the cell membrane and/or cytoplasm, whereas the positive VEGF expression was indicated by brownish yellow particles in the cytoplasm. The specimens were manually scored according to the number of positively staining cells and the staining intensity. 1) The scores were according to the percent of positive cells: 0% = 0 points; ≤25% = 1 point; 26 to 50% = 2 points; ≥50% = 3 points. 2) The scores were evaluated on the basis of staining intensity: absence of positively staining cells = 0 points; light brown coloration in the positive tissues = 1 point; brown coloration with an unstained background or dark brown with light brown background in the positive tissues = 2 points; dark brown with unstained background in the positive tissues = 3 points. The double-blind method of scoring was adopted, and the final score was calculated by multiplying the respective scores: 0 points = scoring 0 points; 1 point = scoring 1 to 2 points; 2 points = scoring 3 to 4 points; 3 points = scoring 6 to 9 points. Negative was a score of 0 to 1 point; positive was a score of 2 to 3 points (Segerman et al., 2003).

Positive CD105 expression was indicated by brownish yellow coloration in the cytoplasm of vascular endothelial cells. The number of capillaries and small blood vessels with tumor staining were calculated according to the microvascular count standard by Weidner et al. (1991). One vascular count represents specimens that display at least a single brown endothelial cell or a mass of endothelial cells clearly demarcated from the surrounding tissue. The sections were first observed under low magnification (40X) to choose five areas with the highest MVD. The cells were then counted under high magnification (400X), and the average of five MVD scores were calculated and reported as means ± standard deviation (means ± SD).

The Motic MED 6.0 digital medical image analysis system was used to take photographs, and the Image-Pro Plus image analysis software was used to analyze the images.

Statistical analysis

The SPSS 17.0 software was used for statistical analysis. A chi-square test was used to evaluate the positivity rates of integrin β1 and VEGF expression between the experimental group and the control group. The difference in MVD count between the two groups was assessed using a *t*-test. The results of the integrin β1 and VEGF image analysis, as well as the MVD count, for the two groups are reported as means ± standard deviations (means ± SD). The statistical significance of the relationship between the three factors and the clinical

characteristics was assessed using a *t*-test. A chi-square test was used to evaluate the relationship between MVD-CD105 count and the clinical characteristics. The correlation coefficients between the three factors were determined using a Pearson rank correlation test. Differences with P values less than 0.05 were considered to be significant.

RESULTS

Expression of integrin β1, VEGF, and MVD-CD105

Positive integrin β1 expression displays brownish yellow particles mainly in the cytoplasm and rarely in the cell membrane (Figure 1A). The integrin β1 overexpression rate was significantly higher in the hypopharyngeal squamous cell carcinoma group (78.00%) than in normal hypopharyngeal tissues (35.00%), $\chi^2 = 11.724$, P = 0.001. Positive VEGF expression displays brownish yellow particles in the cytoplasm (Figure 1B). The VEGF overexpression rate was significantly higher in the hypopharyngeal squamous cell carcinoma group (74.00%) than in normal hypopharyngeal tissues (30.00%), $\chi^2 = 9.307$, P = 0.002 (Table 1). Positive CD105 expression displays brownish yellow coloration of the vascular endothelium cytoplasm (Figure 1C). The MVD count under CD105 staining was significantly higher in hypopharyngeal squamous cell carcinoma tissues (37.10 ± 5.95) than in normal hypopharyngeal tissues (8.70 ± 3.34), t = 25.245, P = 0.000 (Table 1).

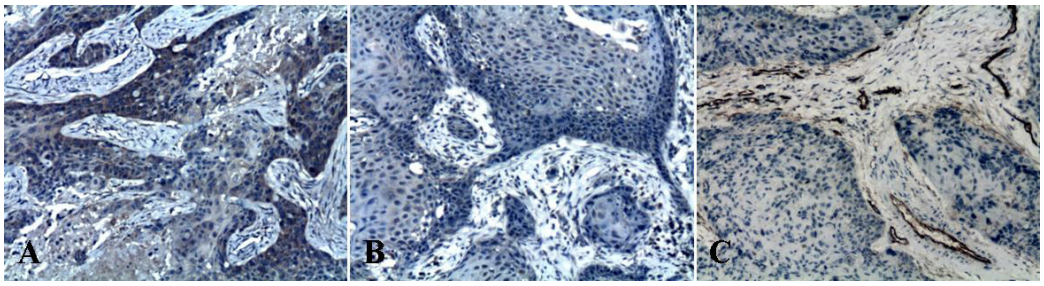

Figure 1. Positive expression of integrin β1, VEGF and MVD-CD105 in the hypopharyngeal squamous cell carcinoma (immunohistochemistry PV-9000 two-step method 100X). **A.** Expression of integrin β1. **B.** Expression of VEGF. **C.** Expression of MVD-CD105.

Table 1. Positive expression of integrin β1, VEGF and value of MVD between experimental and control group.

Index	Positive rate (%)/value of MVD (means ± SD)		χ^2/t	P
	Experimental group	Control group		
Integrin β1	78.00 (39/50)	35.00 (7/20)	11.724	0.001
VEGF	74.00 (37/50)	30.00 (6/20)	9.307	0.002
MVD-CD105	37.10 ± 5.95	8.70 ± 3.34	25.245	0.000

Relationship between the expression of integrin β1, VEGF and MVD-CD105, and clinicopathologic characteristics

The results showed significant differences in pathological grades in terms of T staging and cervical lymph node metastasis (P < 0.05) The results, however, did not show significant differ-

ences in terms of age, gender, history of smoking, and tumor clinical stages (P > 0.05) (Tables 2-4).

Table 2. Relationship between the expression of integrin β1 and clinicopathological characteristics in the hypopharyngeal squamous cell carcinoma.

Clinical characteristics		Case (N)	Protein of integrin β1 value of MVD (means ± SD)	*t*	P
Age	≥60	19	0.2178 ± 0.0783		
	<60	31	0.1847 ± 0.0686	1.571	0.123
Gender	Male	47	0.1959 ± 0.0746		
	Female	3	0.2183 ± 0.0609	0.507	0.614
Smoking	Yes	33	0.2008 ± 0.0756		
	No	17	0.1904 ± 0.0709	-0.474	0.638
T-staging	T1-T2	20	0.1683 ± 0.0571		
	T3-T4	30	0.2166 ± 0.0776	-2.382	0.021
Lymphatic metastasis	Positive	43	0.2069 ± 0.0735		

Table 3. Relationship between the expression of VEGF and clinicopathological characteristics in the hypopharyngeal squamous cell carcinoma.

Clinical characteristics		Case (N)	Protein of VEGF value of MVD (means ± SD)	*t*	P
Age	≥60	19	0.1742 ± 0.0504		
	<60	31	0.1671 ± 0.0534	0.511	0.642
Gender	Male	47	0.1689 ± 0.0523		
	Female	3	0.1832 ± 0.0237	0.458	0.649
Smoking	Yes	33	0.1663 ± 0.0517		
	No	17	0.1766 ± 0.0531	0.660	0.512
T-staging	T1-T2	20	0.1506 ± 0.0487		
	T3-T4	30	0.1826 ± 0.0506	-2.216	0.031
Lymphatic metastasis	Positive	43	0.1764 ± 0.0481		
	Negative	7	0.1289 ± 0.0595	-2.346	0.023
Clinical types*	Pyriform fossa	26	0.1751 ± 0.0701		
	Posterior pharyngeal wall	15	0.1619 ± 0.0194		
	Postcricoid area	9	0.1676 ± 0.0165		>0.05
Histological grades	High differentiation	15	0.1439 ± 0.0453		
	Medium-poor differentiation	35	0.1809 ± 0.0511	-2.418	0.019

*Clinical types are divided according to the tumor primary location. This study uses the *t*-test to evaluate relationship between experimental groups. The difference was not statistically significant with correction test α = 0.05 (P > 0.05).

Table 4. Relationship between the expression of MVD-CD105 and clinicopathological characteristics in the hypopharyngeal squamous cell carcinoma.

Clinical characteristics		Case (N)	Protein of CD105 value of MVD (means ± SD)	*t*	P
Age	≥60	19	36.0526 ± 6.3287		
	<60	31	37.2258 ± 6.3703	0.634	0.529
Gender	Male	47	36.9547 ± 6.0288		
	Female	3	39.3333 ± 4.7258	-0.667	0.508
Smoking	Yes	33	36.8182 ± 5.7088		
	No	17	37.6471 ± 6.5282	-0.463	0.645
T-staging	T1-T2	20	34.2500 ± 5.4664		
	T3-T4	30	39.0333 ± 5.5241	-3.012	0.004
Lymphatic metastasis	Positive	43	31.1429 ± 5.8716		
	Negative	7	38.3721 ± 5.2373	-3.334	0.002
Clinical types*	Pyriform fossa	26	35.7692 ± 6.6711		
	Posterior pharyngeal wall	15	38.9333 ± 5.8244		
	Postcricoid area	9	34.6667 ± 6.3836		>0.05
Histological grades	High differentiation	15	34.1333 ± 6.0576		
	Medium-poor differentiation	35	34.7429 ± 5.2487	-2.717	0.009

*Clinical types are divided according to the tumor primary location. This study uses the *t*-test to evaluate relationship between experimental groups. The difference was not statistically significant with correction test α = 0.05 (P > 0.05).

Relationship between integrin β1, VEGF, and MVD in hypopharyngeal squamous cell carcinoma

The correlation coefficients between the three factors were determined using a Pearson's rank correlation test. VEGF expression was positively associated with the MVD count in hypopharyngeal squamous cell carcinoma ($r = 0.582$, $P < 0.05$). Integrin β1 was not associated with VEGF ($r = 0.247$, $P = 0.084$) or the MVD count ($r = -0.119$, $P = 0.411$).

DISCUSSION

Studies (Hazlehurst et al., 2007; Yao et al., 2007) have shown that the integrin β1 subunit is closely related to apoptosis, proliferation, invasion, and metastasis in a variety of tumors. Integrin is abnormally expressed in head and neck cancer, especially in squamous cell carcinomas. The main function of integrin β1 can be summarized as follows (Takada et al., 2007; Legate et al., 2009): 1) forms the bridge between cells and ECM, intercellular adhesion; 2) transfers intracellular and extracellular information; and 3) mediates neovascularization. When the extracellular region of integrin binds to a ligand, the intracellular region of integrin connects with the cytoskeleton and a variety of signal molecules, causing ECM-integrin interaction with cytoskeletal proteins to mediate communication between cells (Figure 2). In addition, continuous integrin β1 stimulation reduces the expression of intercellular adhesion molecule-1 (ICAM-1), which induces cytotoxic T lymphocyte to kill cancer cells. Cancer cells evade the body's immune surveillance if ICAM-1 is expressed at low levels. Mitogen-activated protein kinase (MAPK) has an important function in intracellular signal transduction. Integrin β1, a tyrosine kinase receptor, is activated into a heterodimer that acts on intracellular secondary messengers such as growth factor receptor-bound protein 2 and connexin of Shc, which activate the Ras/Raf/MAPK signaling pathways, enhance the transcriptional activity of oncogenes (such as c-fos, c-myc, and c-jun), stimulate the intracellular signal cascade, activate downstream signal molecules, stimulate cell proliferation, form tumors, and cause cell migration (Riese and Stern, 1998). Moreover, integrin β1 mediates intracellular signaling through phosphoinositide 3-kinase/silk and the threonine kinase pathway (Luo et al., 2003).

Integrin β1 expression was evaluated by immunohistochemistry in 50 specimens of hypopharyngeal squamous cell carcinoma and 20 specimens of normal hypopharyngeal tissues. Our results showed that the integrin β1 expression rate was significantly higher in hypopharyngeal squamous cell carcinoma (78.00%) than in normal hypopharyngeal tissues (35.00%, $\chi^2 = 11.724$, $P = 0.001$). This observation suggests that integrin β1 overexpression may indicate the occurrence of hypopharyngeal squamous cell carcinoma.

Furthermore, integrin β1 expression did not significantly differ in terms of age, gender, history of smoking, and tumor clinical stages ($P > 0.05$). Integrin β1 expression was statistically different ($P < 0.05$) between different pathological grades, T staging, and incidence of cervical lymph node metastasis. Hence, integrin β1 may be related to the invasion and metastasis of hypopharyngeal squamous cell carcinoma. Patient prognosis may be influenced by this finding, which may be one of the reference indicators for assessing prognosis.

Park et al. (2006) found that integrin β1 is abnormally expressed in breast cancer tissue, and anti-integrin β1 antibodies decreased or increased apoptosis in four breast cancer cell

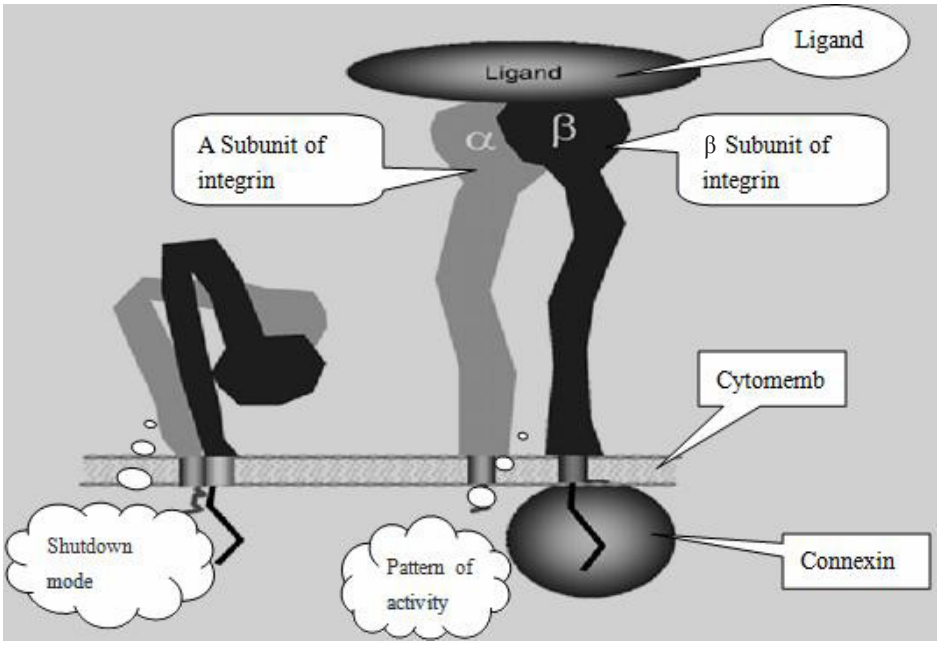

Figure 2. Simple structural pattern of integrin (including: shutdown mode with low affinity, pattern of activity with high affinity).

lines. Integrin β1 inhibits tumor cell proliferation and induces apoptosis through integrin-mediated calcium influx, changes in the expression apoptosis related-gene, phosphorylation, and proliferation (Ruoslahti and Reed, 1994). During the tumorigenesis of hypopharyngeal squamous cell carcinoma, excessive integrin β1 expression phosphorylates the apoptosis-related protein BAD (Bcl-xL/Bcl-2 associated death promoter), and restrains BAD-regulating anti-apoptotic protein Bcl-2 and Bcl-xl, which leads to apoptosis or uncontrolled growth of some cells. Integrin β1 mediates two-way transmission of information inside and outside the cell. The main pathways include the G-protein pathway and the enzyme-linked receptor pathway through which the signal is transmitted to hypopharyngeal tissue, resulting in abnormal hypopharyngeal cell differentiation and formation of hypopharyngeal malignancies. Integrin β1 can also adjust cellular calcium influx and activate calpain activator to influence the information transmission inside and outside of the cell. Another major function of integrin β1 is mediating intercellular adhesion between cells through ECM-integrin interaction with cytoskeletal proteins, which connects cancer cells and adjacent normal cells. When hypopharyngeal cancer cells are closely connected with the surrounding tissue through integrin β1 mediation, the signal is transmitted though the GJIC pathway, resulting in abnormal differentiation, growth of adjacent tissue, and invasion and proliferation of squamous cell carcinoma. Integrin β1 reduces the expression of ICAM-1 and prevents the adhesion of cytotoxic T lymphocytes onto cancer cells, such that the cancer cells avoid being killed by the immune system. We speculate that integrin β1 has a major effect on the occurrence, growth, and invasion of hypopharyngeal squamous cell carcinomas through the aforementioned pathways. Cell adhesion and cell signaling interactions induce cancer tissue growth and proliferation. The detailed aspects of

associated factors and specific mechanisms need further research at the molecular level. We hypothesized that the integrin β1 expression and distribution can be used to determine the prognosis and therapeutic value in hypopharyngeal cancers. Anti-integrin β1 antibodies can also be used to inhibit integrin β1 expression, such that the role of drugs in the tumor cells may be mediated or enhanced, reducing tumor resistance. The prospects of integrin β1 are broad, and we still have to continue comprehensive and in-depth studies.

VEGF is the most important factor that stimulates angiogenesis. VEGF is highly expressed in many tumor tissues, and it is closely related to the occurrence, development, invasion, and metastasis of tumors (Joo et al., 2003). VEGF promotes the proliferation of endothelial cells, boosts the generation of vascular support, increases vascular permeability, changes the extracellular matrix, promotes lymphangiogenesis and metastasis, restrains tumor cell apoptosis, induces monocyte tropism and migration, and regulates monocyte homeostasis. VEGF increases vascular permeability, resulting in swelling of the ECM, and promotes angiogenesis, resulting in differentiation through large amounts of fibrous proteins. VEGF also allows endovascular cancer cells to easily penetrate the vascular fundus (Weidner et al., 1992). In addition, VEGF significantly prolongs the lifespan of vascular endothelial cells, increasing endothelial cell reproduction by 15 to 20 times.

Petruzzelli et al. (1997) used an enzyme-linked immunosorbent assay (ELISA) to detect VEGF expression in five different types of head and neck tumors, and the results showed significant VEGF expression. Moreover, the proliferation of endothelial cells was significantly inhibited in the cell lines treated with VEGF antibodies. These observations suggest that VEGF is related to endothelial cell mitosis in head and neck tumors.

VEGF stimulates the growth of endothelial cells and mediates neovascularization to provide hypopharyngeal squamous cell carcinomas with nutrients and oxygen, as well as allowing cancer cells to penetrate the neovascular fundus, to enter the bloodstream, distribute into neighboring and distant organs, form tumors, infiltrate, and cause distant metastasis. The study confirmed (Tsurusaki et al., 1999; Kitadai et al., 2001) that VEGF also induces the formation of lymphatic vessels and mediates the entrance of cancer cells into the lymphatic circulation, resulting in lymph node metastasis.

The present study found that VEGF expression is significantly higher in hypopharyngeal squamous cell carcinomas (74.00%) than in normal hypopharyngeal tissues ($P = 0.001$). We also used an image analysis system to analyze the VEGF protein expression levels in hypopharyngeal squamous cell carcinomas and found that the MVD count did not significantly differ in terms of age, gender, history of smoking, and tumor clinical stages ($P > 0.05$). These results are consistent with those derived from analysis of VEGF expression in head and neck cancer by *in situ* hybridization and immunohistochemistry, which suggests that VEGF is closely related to the occurrence and development of the hypopharyngeal squamous cell carcinoma. VEGF likely promotes endothelial cell proliferation and the generation of vascular support, increases vascular permeability, provides nutritional foundation for the growth of the hypopharyngeal squamous cell carcinoma, and promotes lymphangiogenesis and metastasis, which are associated with poor prognosis. The exact mechanism needs further research. Hence, determining the VEGF expression levels in hypopharyngeal squamous cell carcinomas is very important for the early diagnosis and detection of this disease.

Angiogenesis is the most basic requirement for tumors to obtain nutrition, survive, and form distant metastasis. MVD is a reliable indicator that reflects the activity of tumor angiogenesis, and it is closely related to tumor differentiation and metastasis (Foote et al., 2005).

Through vascular markers, such as CD31, CD34, CD105, vWF, and VIII, MVD can indicate the quantity, shape, and distribution of neovascularization in tumor tissues. MVD can be used to quantify the speed of blood vessel growth and reflect oxygen supply and nutritional support of the tumor tissue.

The results of numerous similar studies confirm that CD105 is the most specific indicator for immature blood vessels in tumor tissues, accurately reflecting the tumor MVD.

The present study found that the MVD count did not significantly differ in terms of age, gender, history of smoking, and tumor clinical stages in the hypopharyngeal squamous cell carcinoma ($P > 0.05$). The MVD count was significantly higher in the T_3 to T_4 group than in the T_1 to T_2 group of hypopharyngeal squamous cell carcinoma tissues [$t = -3.012$ (u), $P = 0.004 < 0.05$]. The MVD count was significantly higher in the well-differentiated group than in the moderately to poorly differentiated group [$t = -2.717$ (u), $P = 0.009 < 0.05$], and was significantly higher in the lymphatic metastasis group than in the non-lymphatic metastasis group [$t = -3.334$ (u), $P = 0.002 < 0.05$]. Therefore, MVD-CD105 is closely related to the occurrence, growth, and metastasis of hypopharyngeal tumors. The test results will help in the early diagnosis and prognostication of hypopharyngeal squamous cell carcinomas.

In the hypopharyngeal squamous carcinoma tissues, MVD revealed by CD105 staining showed two trends. In the poorly differentiated group, the capillaries lay in cancerous tissue. However, in the well-differentiated group, the capillaries were inside nests and/or in the cancer interstitial area. To some extent, this distribution may help tumor cells enter the bloodstream, accelerate tumor cell growth, and enhance invasiveness. We did not intensively describe the reason for the differences in microvascular distribution for the various hypopharyngeal squamous cell carcinoma but we will further explore the specific mechanisms that lead to this phenomenon in future studies.

Saad et al. (2003) demonstrated that CD105 is better than CD34 and vWF as tumor endothelial markers and predictor of tumor prognosis. CD105 is an ideal marker for angiogenesis. In addition, CD105 expression is induced by both hypoxia and TGF-β1. During the early rapid growth stages, tumor tissues, especially malignant tumors, need plenty of oxygen and nutrients, but the normal growth of blood vessels and the formation rate do not meet these needs. CD105 is overexpressed during active angiogenesis in endothelial cells through the aforementioned mediation model, which also explains why CD105 is modestly expressed or absent in the endothelial cells of large blood vessels and normal tissues (Li et al., 2000).

The morphology, change in quantity, and distribution of new blood vessels react to changes in the biological behavior of the tumor tissues. We consider the MVD of hypopharyngeal squamous cell carcinomas to be closely related to disease progression based on MVD expression, which is consistent with clinical progression and histologic differentiation of tumors. To some extent, it can be used to quantify the new blood vessels in tumors and we speculate that MVD can be used as a quantitative indicator for evaluating hypopharyngeal squamous cell carcinomas.

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