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Original Research Article

Relationship between integrin αvβ3 and α6β1 expression levels and clinicopathological characteristics of cervical cancer

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Abstract

Purpose: *The study was aimed at investigating the relationship between integrin αvβ3 and α6β1 expression levels and clinicopathological characteristics of cervical cancer.*
Methods: *A total of 78 cases of pathologically confirmed cervical cancer patients admitted to our hospital from April 2012 to September 2013 were selected. The expression levels of integrin αvβ3 and α6β1 in the cervical cancer tissue (study group, n=78) and adjacent normal tissue (control group, n=49) were detected by immunohistochemistry. Then, the relationship between the expression of two indicators in cervical cancer tissues and the clinicopathological features of patients were analyzed.*
Results: *Positive expression rate of integrin αvβ3 in cervical cancer tissues was significantly higher than that in adjacent normal tissues (73.08% vs 2.04%, P<0.05). For integrin α6β1, the positive expression rate in cervical cancer tissues was significantly higher than that in adjacent normal tissues (67.95% vs 8.16%, P<0.05). The expression level of integrin αvβ3 in cervical cancer tissues with different degree of differentiation, tumor diameter, depth of invasion, and lymph node metastasis was significantly different (P<0.05). Integrin α6β1, the expressed in cervical cancer tissues with significantly different degrees of differentiation, infiltration depth and lymph node metastasis (P<0.05).*
Conclusion: *The integrin αvβ3 and α6β1 are highly expressed in cervical cancer tissue. The expression level of integrin αvβ3 was related to the differentiation degree, diameter of tumor, infiltration depth and lymph node metastasis, while the expression of integrin α6β1 was related to the clinicopathological features, such as the differentiation degree of cancer tissues, depth of invasion, and lymph node metastasis.*

Key words: *Cervical cancer, Clinicopathological characteristics, Integrin α6β1, Integrin αvβ3.*

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INTRODUCTION

Cervical cancer is a common malignant tumor of the reproductive system in females, and the mortality rate ranks first in cancer mortality in some developing countries [1]. Symptoms of cervical cancer include contact vaginal bleeding, abnormal vaginal discharge, and other secondary manifestations with the metastasis and invasion of the tumor [2]. At present, various screening

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methods have improved the early diagnosis and significantly reduced mortality rate of cervical cancer. As a malignant tumor, the occurrence, invasion and metastasis of cervical cancer are associated with multiple factors, but the molecular mechanism of a series of biological behaviors of cervical cancer cells is still unclear [3]. Also, the occurrence of cervical cancer is associated with high-risk of human papilloma-virus infection and a variety of primary and tumor suppressor genes, but its specific pathogenesis has not been fully defined [4]. Exploring the association between cervical cancer factors and its various clinical features is a key towards clarifying the developmental mechanism of cervical cancer and provide a new direction for its treatment.

Integrins are a kind of surface receptor proteins that are expressed on the surface of various tumor cells and are expected to be targets for the treatment of cancer [5]. Integrin αvβ3 and α6β1, as important members of the integrin family, exert physiological roles in regulating apoptosis and cell adhesion, and function in cancer invasion and metastasis [6,7]. Integrin αvβ3 is one key member of the integrin family, and its specific binding with osteopontin and vitronectin can promote extracellular matrix decomposition, maintain vascular integrity and angiogenesis [8]. Integrin αvβ3 interacts with osteopontin to activate the nuclear factor-κB signaling pathway, inhibits apoptosis, and up-regulates the secretion of matrix metalloprotei-nase. In addition, integrin αvβ3 binding with vitronectin accelerates proliferation, migration of vascular smooth muscle cells and maintains capillary wall integrity.

The purpose of the present study was to investige the relationship between the expression levels of integrin αvβ3 and α6β1 in cervical cancer tissues and pathological features.

EXPERIMENTAL

Subjects

A total of 78 patients with cervical cancer admitted to the People's Hospital, Hubei Jingmen, China from April 2012 to September 2013 and confirmed by pathological examination were selected for the study. Inclusion criteria were: (1) complete medical records; (2) no serious cardiovascular and cerebrovascular diseases; (3) no anti-tumor treatment such as surgery, radiotherapy, chemotherapy, targeted therapy before treatment. Exclusion criteria were: (1) not cooperative in treatment; (2) combined with severe mental disorders; (3) combined with systemic infectious diseases. The cervical cancer tissues resected from 78 patients were collected as the study group, the average age was 56.74±10.21 years old, and 49 cases of adjacent normal tissues confirmed by pathological examination were collected as the control group, with an average age of 57.29±10.37 years old. This study was approved by the Medical Ethics Committee of People's Hospital, Hubei Jingmen, China and written informed consent was obtained from subjects or their family members before treatment.

Immunohistochemical analysis

Excised cervical cancer tissues and adjacent normal tissues were soaked and fixed with neutral formaldehyde (Beijing Baiao Laibo Technology Co., Ltd.) within 1 h *in vitro*, and the tissues were dehydrated and transparently handled in turn, and the tissues were wrapped with paraffin in a block. A tissue slicer (Germany Leica, Type: EXAKT 310CP) was performed to cut it into serial sections of about 4 μm in thickness, followed by rinsing, drying, dewaxing, and catalase blocking. Then, anti-rabbit, anti-human αvβ3 polyclonal antibody (Hubei Aimeijie Technology Co., Ltd.) and rabbit anti-human α6β1 polyclonal antibody (Hubei Aimeijie Technology Co., Ltd.) were added. The preparation was incubated at 4 °C in the refrigerator overnight, rinsed and secondary antibody was added at room temperature for 15 min. It was rinsed again, DAB kit (Shanghai Yida Industrial Co., Ltd.) was applied for color development and hematoxylin (Xiamen Huijia Biotechnology Co., Ltd.) for counterstaining, dehydrated, and sealed with neutral resin after being transparent. The degree of staining and the number of positive cells were observed under a high-power microscope by two experienced pathologists with independent qualifications using a double-blind method. Tumor cell positive rate score: 0 refers to positive cell rate ≤ 5%, 1 represents positive cell rate 6% -25%, 2 indicates positive cell rate 26% -50%, 3 defines positive cell rate 51 %-75%, 4 means positive cell rate 76%-100%. Staining intensity score: 0 represents that tumor cells are not colored, 1 represents light yellow, 2 represents brown, and 3 represents tan. The two scores were totaled, and the final score 0-2 is negative, and >2 is positive.

Measurements

The levels of expression of integrin αvβ3 and α6β1 in cervical cancer and adjacent normal tissues were measured. Also, the relationship between the expression levels of αvβ3 and α6β1 and pathological features such as age, tumor differentiation, tumor diameter, infiltration degree and lymph node metastasis were analyzed.

Statistical analysis

Statistical analysis was performed with SPSS 22.0 for windows (SPSS Inc., Chicago, IL, USA). Categorical data was analyzed with Chi-square test. $P < 0.05$ was defined as statistically significant.

RESULTS

Expression of αvβ3 and α6β1 in cervical cancer and adjacent normal tissues

The results of immunohistochemistry (Figure 1 and Figure 2) showed that the integrin αvβ3 and α6β1positive particles were yellow and mainly located in the cell membrane and cytoplasm. Positive expression rate of integrin αvβ3 was 73.08% in the cervical cancer tissues and a significantly lower value of 2.04% in the adjacent normal tissues (P<0.05). The positive expression rate of integrin α6β1 in the cervical cancer tissues was 67.95%, while a significantly lower value of 8.16% was recorded for the adjacent normal tissues (P<0.05) (Table 1).

The relationship between the expression levels of αvβ3 and α6β1 and age

The results obtained for the expression levels of αvβ3 and α6β1 in relation to age are presented in Table 2. There was no significant difference in the positive expression rates of integrin αvβ3 (≤60 years was 35 and >60 years was 22) and α6β1 (≤60 years was 34 and >60 years was 19) in cervical cancer tissues of patients of different ages (P>0.05).

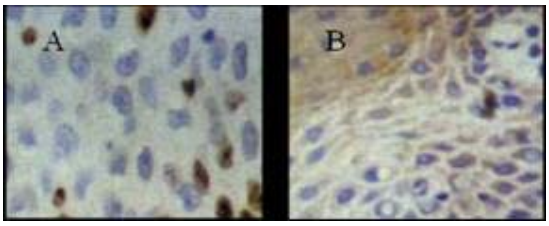

Figure 1: Expression of integrin αvβ3 in cervical cancer tissues and adjacent normal tissues. *A: positive expression of integrin αvβ3 in cervical cancer tissues; B: negative expression of integrin αvβ3 in normal tissues.*

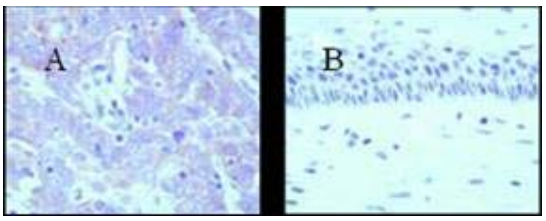

Figure 2: Expression of integrin α6β1 in cervical cancer tissues and adjacent normal tissues. *A: positive expression of integrin α6β1 in cervical cancer tissues; B: negative expression of integrin α6β1 in normal tissues*

The relationship between the expression levels of αvβ3 and α6β1 and differentiation degree of cancer tissues

The positive expression rates of αvβ3 and α6β1 in cancer tissues of patients with different differentiation degrees were significantly different (P<0.05), as shown in Table 3. In the high differentiation and middle/ low differentiation of tumor tissues, the expression rates of αvβ3 were 6 and 51, respectively, while that of α6β1 were 8 and 45, respectively.

Table 1: Comparison of expression levels of αvβ3 and α6β1 in cervical cancer tissues and adjacent normal tissues (n)

Groups	αvβ3		α6β1	
	Positive	Negative	Positive	Negative
Study group (n=78)	57	21	53	25
Control group (n=49)	1	48	4	45
χ^2	61.203		43.482	
P	<0.001		<0.001	

Table 2: Expression levels of αvβ3 and α6β1 in relation to age (n)

Age (Years)	αvβ3		α6β1	
	Positive	Negative	Positive	Negative
≤60	35	14	34	15
>60	22	7	19	10
χ^2	0.182		0.125	
P	0.67		0.723	

Table 3: Expression levels of αvβ3 and α6β1 in relation to the degree of tumor tissue differentiation (*n*)

Degree of differentiation	αvβ3		α6β1	
	Positive	Negative	Positive	Negative
high differentiation	6	11	8	9
Middle/ low differentiation	51	10	45	16
χ^2	0.723		4.356	
P	<0.001		0.037	

Table 4: Expression levels of αvβ3 and α6β1 in relation to tumor diameter and the infiltration degree of the patient's tumor (*n*)

Pathological features	αvβ3		α6β1	
	Positive	Negative	Positive	Negative
Tumor diameter				
≤4 cm	38	19	39	18
>4 cm	19	2	14	7
χ^2	4.422		0.022	
P	0.036		0.883	
Infiltration depth				
≤1/2 full thickness	15	16	17	14
＞1/2 full thickness	42	5	36	11
χ^2	15.94		4.06	
P	<0.001		0.044	

Table 5: Expression levels of αvβ3 and α6β1 in relation to lymph node metastasis (*n*)

Lymph node metastasis	αvβ3		α6β1	
	Positive	Negative	Positive	Negative
yes	33	5	31	7
no	24	16	22	18
χ^2	7.136		6.321	
P	0.008		0.012	

The relationship between the expression levels of αvβ3 and α6β1 and the diameter and infiltration of tumors

The positive expression rate of integrin αvβ3 in cancer tissues with different tumor diameters was significantly different (*P*<0.05) as shown in Table 4. In tumor diameter of ≤4 cm, the positive expression rate of integrin αvβ3 was 38, while its expression in the >4 cm tumor diameter was 19. However, there was no significant difference in the positive expression rate of αvβ3 and α6β1 in the ≤4 cm tumor diameter unlike in patients with different infiltration depths (Table 4) (P<0.05). The expression levels of αvβ3 in the ≤1/2 full thickness and ＞1/2 full thickness were 15 and 42, respectively, while the expression levels of α6β1 were 17 and 36, respectively.

The relationship between the expression levels of αvβ3 and α6β1 and lymph node metastasis

The positive expression rates of αvβ3 and α6β1 in cancer tissues with different lymph node metastasis were significantly different (*P*<0.05) (Table 5).

DISCUSSION

Cervical cancer is a very common gynecological malignancies, and the incidence rate in young women is increasing in recent years [9]. Investigation of the mechanism of cervical cancer development from the perspective of molecular science is of great significance for its treatment. Integrins are a species of cell adhesion factors that are extensively distributed on the surface of endothelial cells, macrophages, smooth muscle cells, and tumor cell membranes.

As a member of the membrane receptor family, the main physiological function of integrin is to regulate cell differentiation, proliferation and apoptosis by mediating intercellular signaling [10, 11]. The integrin αvβ3 (vitronectin receptor) has been reported to be associated with pathophysiology of malignant tumors [12]. Upheber *et al* [13] found that integrin αvβ3 was highly expressed in ovarian cancer tissues, and can promote tumor development and metastasis.

Integrin α6β1 is the most potent signaling molecule in the integrin family, and plays a role in promoting tumor cell differentiation, proliferation and metastasis by binding to cluster of

differentiation 151 (CD151) [14]. CD151 is the only oncogene in the four transmembrane protein superfamily. A stable complex formed through binding CD151 and integrin α6β1 enhances the physiological function of integrin α6β1, reduces fibronectin junction, prompts cell movements on laminin, and promotes cell migration [15]. In addition, integrin α6β1 can also weaken the immune surveillance and immune defense functions of the immune system on tumor cell, thereby further accelerating tumor growth and reducing tumor cell apoptosis [16].

In the present study, the expression levels of integrin αvβ3 and α6β1 in cervical cancer tissues were immunohistochemically analyzed. In our finding, the expression level of integrin αvβ3 was significantly (P<0.05) higher than that in adjacent normal tissues, which coincided with the results of Upheber *et al*. [15]. The expression level of integrin αvβ3 in cervical cancer tissues was significantly different in patients with different differentiation degrees, tumor diameter, depth of invasion and lymph node metastasis, suggesting that the expression of integrin αvβ3 is associated with pathological features such as degree of tumor differentiation, tumor diameter, depth of invasion and lymph node metastasis.

On the other hand, expression of integrin α6β1 in cervical cancer tissues was higher than that in adjacent normal tissues, and the expression level of integrin α6β1 in cervical cancer tissues was significantly (P<0.05) different in patients with different cancer differentiation degrees, invasion depth and lymph node metastasis. This observation suggested that the expression of integrin α6β1 is associated with the pathological features such as differentiation degree, depth of invasion and lymph node metastasis.

CONCLUSION

In this study, the results obtained indicated that the expression levels of integrin αvβ3 and α6β1 in cervical cancer tissues showed an upward trend. The expression level of integrin αvβ3 was related to pathological features such as tumor differentiation, tumor diameter, depth of invasion and lymph node metastasis, while expression of integrin α6β1 was associated with pathological features such as the degree of differentiation, invasion depth and lymph node metastasis

DECLARATIONS

Acknowledgement

None declared.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. All authors read and approved the manuscript for publication.

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