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Original Article

Correlation analysis of integrin αvβ3, T-cadherin, and VEGF expression in gastric cancer tissue with microangiogenesis

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Abstract: This study was designed to analyze the relationship between integrin αvβ3, T-cadherin, and VEGF expression with microvessel density (MVD) and clinicopathological characteristics of gastric cancer. A total of 68 gastric cancer patients and 30 healthy volunteers were enrolled. Serum T-cadherin and VEGF levels were tested by ELISA. Integrin αvβ3, T-cadherin, VEGF, and CD34 expression in gastric cancer tissues and adjacent normal tissues was detected by immunohistochemistry. The relationship between integrin αvβ3, T-cadherin, VEGF, and CD34 expression with MVD and clinicopathological characteristics was analyzed. Serum VEGF level was significantly higher, while T-cadherin was lower in gastric cancer patients compared to those in the normal control group ($P < 0.05$). Positive rates of integrin αvβ3 and VEGF were higher, MVD counting was larger, and T-cadherin positive rate was lower in gastric cancer tissues than those in the adjacent normal controls ($P < 0.05$). αvβ3 positive expression was correlated with differentiation, infiltration depth, lymph node metastasis, and TNM staging. VEGF positive expression was related to lymph node metastasis and TNM staging. T-cadherin and CD34 positive expression was associated with differentiation, lymph node metastasis, and TNM staging ($P < 0.05$). αvβ3 and VEGF expression showed a positive correlation ($r = 0.53$ and 0.69, respectively; $P < 0.05$), whereas T-cadherin exhibited a negative correlation with MVD ($r = -0.51$, $P < 0.05$). VEGF was positively correlated with αvβ3 ($r = 0.58$, $P < 0.05$), while it was negatively correlated with T-cadherin ($r = -0.49$, $P < 0.05$). Abnormal expression of integrin αvβ3, T-cadherin, and VEGF was associated with gastric cancer progression and microangiogenesis.

Keywords: Gastric cancer, integrin αvβ3, T-cadherin, VEGF

Introduction

Gastric cancer is a common clinical malignancy with high mortality and morbidity. Due to hidden clinical symptoms, early screening for gastric cancer is poor. Most patients are diagnosed in the late stage, which is combined with distant metastasis [1, 2]. Distant metastasis of gastric cancer includes liver and extrahepatic metastasis. Liver metastasis is a multi-step complex process involving multiple factors, such as angiogenesis, extracellular matrix metalloproteinase, adhesion molecules, and other factors [3, 4]. At present, the etiology and pathogenesis of gastric cancer have not been fully elucidated. Angiogenesis plays an important role in the development and progress of malignant tumors. The balance of angiostatin and angiogenic factors is important in the regulation of angiogenesis. Tumor angiogenesis is a complex interactive process between vascular endothelial cells and tumor cells, including new basement membrane and vascular ring formation, endothelial matrix membrane dissolution, and endothelial cell migration and proliferation [5, 6].

The initiation of tumor angiogenesis is the degradation of extracellular matrix. Matrix metalloproteinase-2 (MMP-2) participates in basal membrane degradation process by degrading the basement membrane and extracellular

Table 1. Serum T-cadherin and VEGF comparison

Group	Cases	T-cadherin (mg/ml)	VEGF (ng/L)
Gastric cancer	68	1.59 ± 0.08*	168.95 ± 21.13*
Control	30	2.52 ± 0.09	20.11 ± 6.77

*$P < 0.05$, compared with control.

matrix structural protein. Vascular endothelial growth factor (VEGF) plays a critical role in vascular regulatory factors that can specifically stimulate the proliferation of vascular endothelial cells. VEGF overexpression in a variety of tumor tissues is associated with tumor invasion and metastasis. Endostatin suppresses tumor angiogenesis by inhibiting endothelial cell growth and migration [7, 8]. It has been shown that the adhesion molecule E-cadherin can induce tumor angiogenesis, whereas the role of T-cadherin in gastric cancer invasion, metastasis, and tumor angiogenesis is scarcely explored [9, 10]. Integrins, are important adhesion molecules that mediate the interaction between cells and extracellular matrix. Integrins play an important role in tumor angiogenesis by regulating cell adhesion, proliferation, and migration. They are also involved in the process of invasion and metastasis of various malignant tumors. *In vitro* and *in vivo* experiments show that anti-αvβ3 monoclonal antibody can effectively inhibit angiogenesis and inhibit tumor proliferation [11, 12]. This study was intended to analyze the relationship between integrin αvβ3, T-cadherin, and VEGF expression with microvessel density (MVD) and clinicopathological characteristics of gastric cancer.

Materials and methods

General information

A total of 68 gastric cancer patients between Jan 2015 and Oct 2016 in the First Hospital of Jingzhou (Hubei, China) were enrolled, including 32 males and 36 females with mean age at 59.1 ± 3.3 (30-76) years old. All the patients were diagnosed by pathology. The gastric tumor was removed by surgery, and the adjacent normal tissue was obtained from the place with more than 5 cm from the tumor edge. No patients received chemotherapy or radiotherapy before operation. Another 30 cases of healthy volunteers that received physical examination in the corresponding period were selected as controls, including 15 males and 15 females with mean age at 60.2 ± 4.2 (30-75) years old. No statistical significance was observed on gender, age, and weight between the two groups ($P > 0.05$). All the subjects signed informed consent and the study was approved by the ethics committee in First Hospital of Jingzhou (Hubei, China).

Reagents and instruments

VEGF and T-cadherin ELISA kits were provided by Jiancheng Bioengineering Institute (Nanjing, China). VEGF, αvβ3, and T-cadherin antibodies, SP immunoassay kit, CD34 monoclonal antibody, and protein quantification kit were supplied by Boster (Wuhan, China). Inverted microscope was obtained from Olympus (Japan). Tissue embedder was purchased from SAKURA (Japan). Slicer was bought from Leica (Germany). Oscillator was obtained from Jinghong (Shanghai, China). High-temperature resistance plastic dyeing frame was obtained from Maxim (Fuzhou, China). Computer image analysis system was provided by Hp (USA).

Methods

ELISA: The venous blood was extracted and centrifuged to obtain the supernatant. Serum T-cadherin and VEGF contents were tested by ELISA. The plate was read at 450 nm to obtain the absorbance value. A linear regression equation was established to calculate concentration.

Immunohistochemistry: The tissues were fixed by formalin and embedded after dehydration and waxing. Next, paraffin sections were toasted and repaired after dehydration. Then the sections were blocked by goat serum and incubated in 50 μL primary antibodies (1:100, 1:200, 1:200, respectively) at room temperature for 1 h. Next, the sections were added with 50 μL secondary antibody (1:100, 1:200, 1:200, respectively) for 10 min. The sections were treated by 50 μL streptavidin-peroxidase for 10 min. After coloration, redyeing, and differentiation, the sections were observed under the microscope. CD34 was used to stain new blood vessel endothelial cells by SP method. MVD value was calculated upon one blood ves-

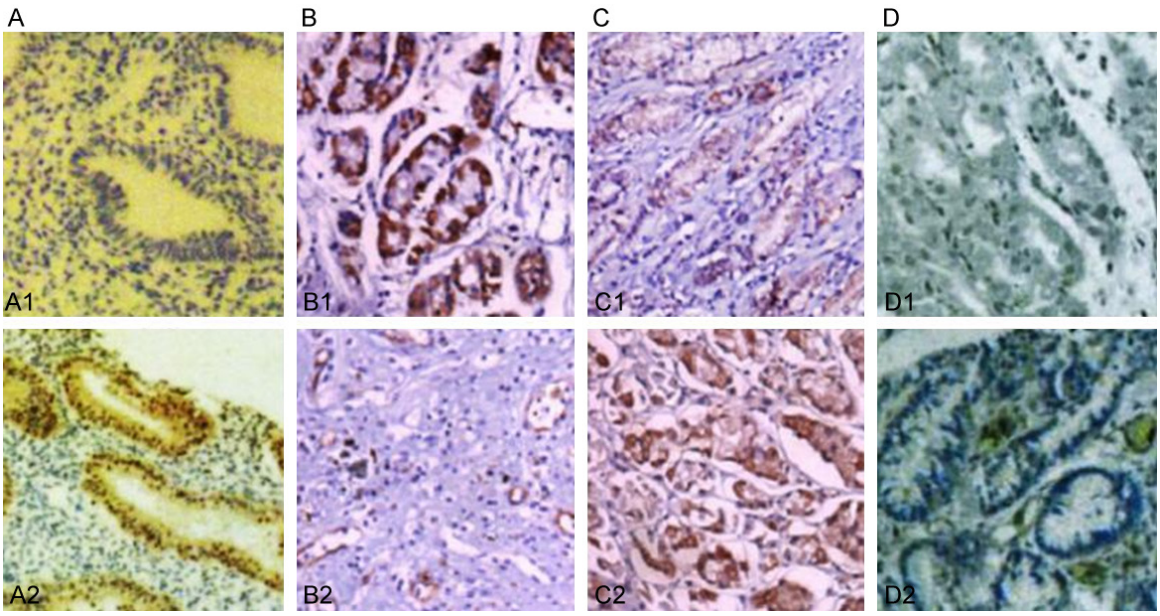

Figure 1. αvβ3, T-cadherin, and VEGF positive expression in gastric cancer tissue (× 100). A. αvβ3; B. T-cadherin; C. VEGF; D. CD34; 1, adjacent normal tissue; 2, gastric cancer tissue.

Table 2. αvβ3, T-cadherin, and VEGF positive expression and MVD count in gastric cancer

Tissue	αvβ3 (%, n)	T-cadherin (%, n)	VEGF (%, n)	MVD (/HPF)
Gastric cancer tissue	58.82 (40/68)*	44.12 (30/68)*	67.65 (46/68)*	18.25 ± 6.01*
Adjacent normal tissue	20.59 (14/68)	61.76 (42/68)	27.94 (19/68)	7.31 ± 5.07
χ^2/t value	20.762	4.250	21.483	23.277
P value	0.000	0.032	0.000	0.000

*$P < 0.05$, compared with control.

sel counting by claybank staining of endothelial cell.

Positive judgement: The immunohistochemistry result was scored by using a semi-quantitative method. Positive cells were defined as those with claybank particles in the cell membrane or cytoplasm. Positive cell percentage: Score 0, no positive cells; Score 1, positive cells < 25%; Score 2, positive cells between 25% and 50%; Score 3, positive cells between 50% and 75%; Score 4, positive cells ≥ 75%. Staining intensity: score 0, no staining; Score 1, light yellow; Score 2, claybank; Score 3, sepia. Staining score = cell staining intensity × positive cell percentage. Negative, score 0-3. Weak positive (+), score 4-8. Positive (++), score 9-12.

Statistical analysis

All data analyses were performed on SPSS 19.0 software. The enumeration data were analyzed by chi-square test. Measurement data were presented as mean ± standard deviation and compared by ANOVA. Correlation analysis was performed by Spearman. P < 0.05 was depicted as statistical significance.

Results

Serum T-cadherin and VEGF levels in the gastric cancer patients

Serum VEGF level was significantly higher, while T-cadherin was lower in gastric cancer patients than those in the normal control ($P < 0.05$, **Table 1**).

αvβ3, T-cadherin, and VEGF positive expression in gastric cancer tissue and MVD counting

The positive rates of integrin αvβ3 and VEGF were higher, MVD counting was larger, and T-cadherin positive rate was lower in gastric cancer tissue than those in the adjacent nor-

Table 3. Correlation analysis of αvβ3, T-cadherin, and VEGF positive expression with clinicopathological characteristics in gastric cancer

Item	N	αvβ3 positive rate	T-cadherin positive rate	VEGF positive rate	MVD (/HPF)
Gender					
Male	32	19 (59.37)	14 (43.75)	22 (68.75)	18.44 ± 5.21
Female	36	21 (58.33)	16 (44.44)	24 (66.67)	18.08 ± 4.66
χ^2/t value		0.070	0.033	0.036	0.380
P value		> 0.05	> 0.05	> 0.05	> 0.05
Age					
< 60	46	27 (58.69)	18 (39.13)	31 (67.39)	18.05 ± 4.99
≥ 60	22	13 (59.09)	12 (54.54)	15 (68.18)	18.67 ± 5.12
χ^2/t value		0.012	1.434	0.042	0.782
P value		> 0.05	> 0.05	> 0.05	> 0.05
Tumor size (cm)					
< 5	25	14 (56.00)	10 (40.00)	14 (56.00)	17.25 ± 4.78
≥ 5	43	26 (60.47)	20 (46.51)	32 (74.42)	18.83 ± 5.17
χ^2/t value		0.113	1.809	2.450	1.056
P value		> 0.05	> 0.05	> 0.05	> 0.05
Pathology type					
Adenocarcinoma	49	30 (61.22)	23 (46.94)	34 (69.39)	18.56 ± 4.78
Other type	19	10 (52.63)	7 (36.84)	12 (63.16)	17.45 ± 4.55
χ^2/t value		0.417	0.824	0.243	1.172
P value		> 0.05	> 0.05	> 0.05	> 0.05
Differentiation					
Poor	47	33 (70.21)	25 (53.19)	34 (72.34)	21.34 ± 5.16
Well-moderate	21	7 (33.33)	5 (23.81)	12 (57.14)	11.33 ± 4.11
χ^2/t value		9.192	5.083	1.532	10.558
P value		< 0.05	< 0.05	> 0.05	< 0.05
Infiltration depth					
Non-serosa infiltration	18	2 (11.11)	4 (22.22)	9 (50.00)	16.63 ± 4.08
Serosa infiltration	50	38 (76.00)	26 (52.00)	37 (74.00)	18.83 ± 5.11
χ^2/t value		19.903	3.118	3.483	1.708
P value		< 0.05	> 0.05	> 0.05	> 0.05
Lymph node metastasis					
No	16	1 (6.25)	1 (6.25)	5 (31.25)	9.15 ± 3.24
Yes	52	39 (75.00)	29 (55.77)	41 (78.85)	21.05 ± 6.02
χ^2/t value			12.169	12.665	12.570
P value		< 0.05	< 0.05	< 0.05	< 0.05
TNM staging					
I+II	36	13 (36.11)	6 (16.67)	19 (52.78)	13.63 ± 4.11
III+IV	32	27 (84.37)	24 (75.00)	27 (84.37)	23.45 ± 6.07
χ^2/t value		16.292	23.382	7.728	10.558
P value		< 0.05	< 0.05	< 0.05	< 0.05

mal control ($P < 0.05$). αvβ3 is mainly expressed on the inner side of the cell membrane. VEGF is mainly located in the cytoplasm. T-cadherin was on the cell membrane. CD34 is mostly expressed in the cell membrane and cytop-lasm (**Figure 1** and **Table 2**).

Correlation analysis of αvβ3, T-cadherin, and VEGF positive expression in gastric cancer tissues with clinicopathological characteristics

αvβ3 positive expression was correlated with differentiation, infiltration depth, lymph node

Table 4. Correlation analysis of αvβ3, T-cadherin, and VEGF positive expression with MVD count

Tissue	αvβ3	*P*	T-cadherin	*P*	VEGF	*P*
MVD	0.53	< 0.05	-0.51	< 0.05	0.69	< 0.05
VEGF	0.58	< 0.05	-0.49	< 0.05	-	-

metastasis, and TNM staging. VEGF positive expression was related to lymph node metastasis and TNM staging. T-cadherin and CD34 positive expression were associated with differentiation, lymph node metastasis, and TNM staging ($P < 0.05$) but not tumor size, age, and gender (**Table 3**).

Correlation analysis of αvβ3, T-cadherin, and VEGF positive expression in gastric cancer tissue with MVD

αvβ3 and VEGF expression showed positive correlation (r = 0.53, 0.69, $P < 0.05$), whereas T-cadherin exhibited negative correlation with MVD (r = -0.51, $P < 0.05$). VEGF was positively correlated with αvβ3 (r = 0.58, $P < 0.05$), while negatively correlated with T-cadherin (r = -0.49, $P < 0.05$) (**Table 4**).

Discussion

The invasion and migration of gastric cancer and other malignant tumors depend on tumor angiogenesis. Tumor angiogenesis processes include extracellular matrix degradation, cellular proliferation, and multiple other steps. The proportion of angiogenesis inhibiting factors and promoting factors affects the tumor angiogenesis process. Endostatin is a highly specific endogenous angiogenesis inhibitor, which inhibits tumor angiogenesis by inhibiting eNOS activation to block the VEGF signaling pathway. VEGF can promote vascular endothelial cell division by specifically binding the receptor on the cell membrane to promote angiogenesis and regulate endothelial cell proliferation. It has been suggested that VEGF overexpression in a variety of tumor cell lines and tumor tissues, is associated with tumor invasion, metastasis, and prognosis [13, 14]. VEGF can also promote tumor distant metastasis by promoting lymphatic hyperplasia around the tumor tissue. The recurrence rate of VEGF positive patients is higher than that of negative expression. VEGF blockade therapy significantly reduces the primary tumor volume and decreases the incidence of distant metastasis [15, 16]. αvβ3 is expressed in most malignant tumor tissues, such as colorectal cancer and hepatocellular carcinoma. αvβ3 expression is closely related to tumor angiogenesis, lymph node metastasis, and invasion depth. It has been shown that αvβ3 is highly expressed in esophageal and colorectal cancers, and is positively correlated with tumor infiltration. Blocking αvβ3 in colorectal cancer decreased tumor cell invasion. αvβ6 may affect gastric cancer distant metastasis through the VEGF and PI3K/AKT pathways [17, 18]. The role of αvβ3 expression in gastric cancer has not been investigated.

T-cadherin is different from the classical cadherin molecule. Since there is no transmembrane region, T-cadherin attaches on cell membrane through glycosylphosphatidylinositol molecules, thus playing an important role in the process of tumor angiogenesis and distant migration. It is downregulated in various tumor tissues, such as pancreatic cancer and colorectal cancer, and is closely related to lymph node metastasis [19, 20]. However, studies documenting T-cadherin expression in gastric cancer have been relatively few. In this study, we investigated the correlation between the expression of αvβ3, T-cadherin, and VEGF in gastric carcinoma, and MVD and clinicopathological characteristics, aiming to provide a basis for the clinical treatment and prognosis evaluation of gastric cancer. Serum VEGF level was significantly higher, while T-cadherin was lower in gastric cancer patients compared with normal controls, suggesting that integrin αvβ3, T-cadherin, and VEGF are abnormally expressed in gastric cancer tissue. It was reported that integrin αvβ6 positive expression was related to tumor differentiation, infiltration depth, lymph node metastasis, and TNM staging. αvβ3 is mainly involved in tumor cell invasion and metastasis, and mediates cell adhesion and migration. It is significantly expressed in malignant tumors and is positively correlated with tumor cell invasive ability through mediating the TGF-β1 signaling pathway. The positive expression of VEGF is associated with lymph node metastasis and TNM staging, which is similar to the results of this study [21, 22], suggesting that integrin αvβ3, T-cadherin, and VEGF may promote angiogenesis, invasion, and

distant metastasis. T-cadherin is an endogenous negative regulator that can inhibit cancer cell metastasis. The inactivation of T-cadherin gene in malignant tumors is related to promoter methylation. The specific mechanism of T-cadherin expression in gastric cancer is still unclear. Limited by the sample size, this study only investigated the relationship between the expression of integrin αvβ3, T-cadherin, and VEGF with the progression and angiogenesis of gastric cancer. The related indexes of angiogenesis in gastric cancer still need to be further explored.

Abnormal expression of integrin αvβ3, T-cadherin, and VEGF were associated with gastric cancer progression and microangiogenesis. Combined detection could be used to evaluate gastric cancer treatment and prognosis.

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Disclosure of conflict of interest

None.

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