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Expression and clinical significance of aquaporin-1, vascular endothelial growth factor and microvessel density in gastric cancer

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Abstract

To investigate the expression and clinical significance of aquaporin-1 (AQP1), vascular endothelial growth factor (VEGF) and microvessel density (MVD) in gastric cancer.

A total of 79 gastric cancer patients who were admitted into Beijing Chao-Yang Hospital from January, 2018 to December, 2019 were involved in this study. Tumor specimens and para-cancerous normal tissues (> 2 cm away from the tumor) of all the enrolled patients were collected. Immunohistochemistry were performed to identify the expression of AQP1, VEGF, and MVD and the correlation between AQP1, VEGF, MVD, and clinicopathological parameters was analyzed.

The expression of AQP1, VEGF and MVD in gastric cancer tissue was increased significantly compared with those in para-cancerous tissue ($P < .05$). AQP1, VEGF, and MVD were closely correlated with gastric cancer differentiation, lymph node metastasis, vascular tumor thrombosis and clinical stage ($P < .05$). Spearman correlation analysis showed that AQP1 was positively associated with VEGF expression ($r = 0.497$, $P < .05$). MVD was enhanced in VEGF or AQP1 positive cancer tissues compared with that in VEGF or AQP1 negative tissue ($P < .05$).

Synergistic effect among AQP1, VEGF, and MVD is involved in occurrence and development of gastric cancer.

Abbreviations: AQP1 = aquaporin-1, IHC = Immunohistochemical, MVD = microvessel density, OS = Overall Survival, VEGF = vascular endothelial growth factor.

Keywords: aquaporin-1, gastric cancer, microvessel, vascular endothelial growth factor

1. Introduction

Gastric cancer is 1 of the most common malignant tumors of the digestive tract, with atypical early symptoms and low diagnosis rate.[1] When diagnosed, it is often in the stage of disease progression. The annual mortality rate of gastric cancer in China is 25.21/100000, which is the first cause of death in all kinds of malignant tumors.[2] Some studies have indicated that microvascular formation plays an important role in the process of tumor occurrence, development and invasion.[3–5] In the process of tumor microvascular formation, a variety of promoters and suppressors are needed.[5,6]

There is a kind of specific aquaporin in animals, plants and microorganisms, which is called aquaporin-1 (AQP1). They are widely distributed inmultiple organs of the body to meet the physiological needs of the human tissues, and also play a vital role in the process of cell life activities and apoptosis.[7] It has been reported that AQP1 was closely related to many kinds of tumors, such as breast cancer, bladder cancer, laryngeal cancer and melanoma.[8] De Ieso ML et al found that the positive expression rate of AQP1 in colorectal cancer was higher than that in the para-cancerous tissues, suggesting that AQP1 was closely related to the occurrence and development of colorectal cancer.[9] There is plenty of evidence that increased angiogenesis is an essential step for cancer progression and this also applies for gastric cancer.[10–13] However the results of AVAGAST trial showed that anti- vascular endothelial growth factor (VEGF) treatment failed to improve overall survival (OS). On the other hand AQPs are also known to be associated with angiogenesis and gastric cancer progression. In addition, some studies pointed out that the expression of VEGF and AQP1 in non-small cell lung cancer was higher than that in para-cancerous tissues, and there was a certain correlation.[14,15] It was speculated that AQP1 could promote tumor invasion and metastasis by interacting with VEGF during tumor growth. Microvessel density (MVD) is a direct way to quantify angiogeneis in a specific tissue, albeit technically demanding and representative of the specific anatomic point at the specific time of sampling.

In this study, we investigated the expression and clinical significance of AQP1, VEGF and MVD in gastric cancer. In

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addition, we analyzed the correlation among AQP1, VEGF, MVD with the clinicopathological parameters in gastric cancer, which was aimed to provide reference for individualized treatment and prognosis evaluation of gastric cancer.

2. Materials and methods

2.1. Study patients

A total of 79 patients diagnosed gastric cancer at the Beijing Chao-Yang Hospital from January, 2018 to December, 2019 were enrolled in this study. All patients underwent radical gastrectomy and fresh specimens were collected. Clinical data such as age, gender, tumor size, differentiation type, lymph node metastasis, vascular tumor thrombosis and clinical stage were recorded. The postoperative pathological staging followed the TNM staging criteria of UICC-AJCC gastric cancer (7th edition). All patients provided the informed consent. The Ethics Committee at Beijing Chao-Yang Hospital had approved the using clinical information and surgical tissue specimens in our study (approval number: 20180703). All procedures and ethical standards were done in accordance with the national research committee and/or institutional.

2.2. Inclusion criteria

Patients were enrolled in this study if they met all the following criteria:

(1) patients diagnosed with gastric cancer;
(2) patients underwent radical gastrectomy;
(3) had a complete and detailed clinicopathological data record.

2.3. Exclusion criteria

Patients meeting any of the following criteria were excluded:

(1) patients underwent preoperative radiotherapy and chemotherapy;
(2) patients with other tumors;
(3) any incomplete clinicopathological data.

2.4. Immunohistochemical (IHC) staining

The cancer tissue and the para-cancerous tissue were taken from each case and stored in a −80°C refrigerator in the central laboratory. All the specimens were fixed with 4% paraformaldehyde, dehydrated, transparently treated, paraffin embedded and sectioned continuously (4 μm). After conventional dewaxing and hydration, the 2-step IHC method (SP staining) was adopted. The operation steps were strictly in accordance with the instructions of the kit, DAB staining, hematoxylin re staining, dehydration after drying, transparency and sealing.

AQP1 Rabbit anti human polyclonal antibody, Rabbit anti human VEGF polyclonal antibody, mouse anti human CD34 monoclonal antibody, SP staining kit and concentrated DAB color development kit were all purchased from Wuhan PhD Biotechnology Co., Ltd.

2.5. Criterion of IHC and MVD

AQP1 was positive when the cell membrane was stained as brown yellow; When the cell membrane or cytoplasm was stained yellow or brown, VEGF was positive.If the content of cell membrane or cytoplasm in each section was more than 5%, VEGF positive expression could be determined. MVD: first selected the vascular dense area under the low power mirror (× 40), then selected 3 fields under the high power mirror (× 200), counted the new blood vessels, and finally took the average value as MVD.

2.6. Statistical analysis

SPSS 17.0 statistical software was used for data analysis, mean ± standard deviation ($x \pm s$) was used for measurement data, t test was used for comparison, rate (%) was used for counting data, Spearman was used for inter group correlation analysis, χ^2 test was used for comparison, $P < .05$ was statistically significant.

3. Results

3.1. Patient characteristics

There were 79 patients with gastric cancer, including 49 males and 30 females, with an average age of 57.3 years (28–81 years). Postoperative pathological stages of the patient included 13 cases in stage I, 21 cases in stage II, 38 cases in stage III, and 7 cases in stage IV. 51 cases were with lymph node metastasis, 28 cases without lymph node metastasis. There were 43 cases with positive tumor thrombus and 36 cases with negative vascular tumor thrombus. According to the degree of differentiation: 11 cases were highly differentiated cancer, 24 cases were moderately differentiated cancer, 39 cases were poorly differentiated cancer and 5 cases were undifferentiated cancer.

3.2. The IHC of AQP1, VEGF and MVD in gastric cancer and para-cancerous tissues

To detect the change of AQP1, VEGF, and MVD level in gastric tumorigenesis, we performed IHC assay using gastric cancer and para-cancerous tissues. Figure 1 displayed the positive expression of AQP1, VEGF and MVD in gastric cancer. Among the 79

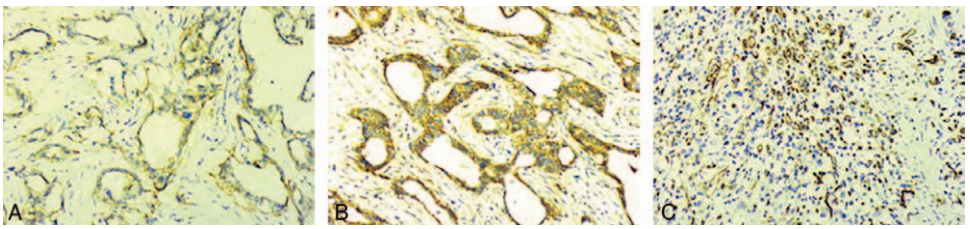

Figure 1. The expression of AQP1, VEGF, and MVD in gastric cancer (200 ×). (A) The positive expression of AQP1 in gastric cancer; (B) The positive expression of VEGF in gastric cancer; (C) The high expression of MVD in gastric cancer. AQP1 = aquaporin-1, MVD = microvessel density, VEGF = vascular endothelial growth factor.

Table 1

Expression of AQP1, VEGF, and MVD in gastric cancer and para-cancerous tissues (N = 79).

Groups	AQP1, n (%)	VEGF, n (%)	MVD (X ± S)
Gastric carcinoma tissues	58 (73.42)	56 (70.89)	41.65 ± 6.10
Para-cancerous tissues	28 (35.44)	30 (37.97)	21.54 ± 5.06
χ^2/t	22.965	17.249	27.606
P value	< .001	< .001	< .001

AQP1 = aquaporin-1, MVD = microvessel density, VEGF = vascular endothelial growth factor.

gastric cancer tissues, AQP1 positive expression was 58 (73.42), VEGF positive expression was 56 (70.89), and the average expression level of MVD was 41.65 ± 6.10.In 79 paracancerous tissues, AQP1 positive expression was 28 (35.44), VEGF positive expression was 30 (37.97), and the average expression level of MVD was 21.54 ± 5.06. The expressions of AQP1, VEGF, and MVD in gastric cancer tissues were significantly higher than those in para-cancerous tissues (both $P < 0.001$) (Table 1).

3.3. The correlation analysis of AQP1 and VEGF in gastric cancer

In gastric cancer tissues with AQP1 negative expression, the positive expression rate of VEGF was 33.33% (7 cases) and the negative expression rate was 66.7% (14 cases). In gastric cancer tissues with AQP1 positive expression, the positive expression rate of VEGF was 84.48% (49 cases) and the negative expression rate was 15.52% (9 cases). Spearman's correlation analysis showed in gastric cancer tissues AQP1 was positively correlated with the expression of VEGF ($rs = 0.497$, $P < 0.05$) (Table 2).

3.4. The comparison of expression of AQP1, VEGF and MVD in gastric cancer

In gastric cancer tissues with AQP1 positive expression, the average expression level of MVD was 44.16 ± 4.52, which significantly higher than the average expression level (34.80 ± 4.44) that in gastric cancer tissues with AQP1 negative expression ($P < 0.001$). The average expression level of MVD in gastric cancer tissues with VEGF positive expression was 44.31 ± 4.65, and in gastric cancer tissues with VEGF negative expression was 35.17 ± 3.98. There was significantly difference between VEGF positive and VEGF negative groups ($P < .001$) (Table 3).

3.5. The correlatio n between the expression of AQP1, VEGF, MVD, and clinicopathological parameters in gastric cancer

In addition, we investigated the correlation between the expression of AQP1, VEGF, MVD and clinicopathological parameters in gastric cancer. Chi-square tests showed that the expression of AQP1 and VEGF were relevant to the differentiation of gastric cancer, lymph node metastasis, vascular tumor thrombus and clinical stage ($P < .05$), while the expressions of AQP1 and VEGF were not related to the size of the tumor ($P > .05$) (Table 4). Table 5 displayed the expression of MVD was related to gastric cancer differentiation, lymph nodes metastasis, vascular tumor thrombus and clinical stage ($P < .05$).

Table 2

Correlation analysis of AQP1, and VEGF in gastric cancer.

	VEGF, n		
AQP1, n	Negative	Positive	Total
Negative	14	7	21
Positive	9	49	58
Total	23	56	79

AQP1 = aquaporin-1, VEGF = vascular endothelial growth factor.

Table 3

The comparison of expression of AQP1, VEGF, and MVD in gastric cancer (X ± S).

Groups	n	MVD	t	*P* value
AQP1				
Positive	58	44.16 ± 4.52	−8.146	<.001
Negative	21	34.80 ± 4.44		
VEGF				
Positive	56	44.31 ± 4.65	−8.260	<.001
Negative	23	35.17 ± 3.98		

AQP1 = aquaporin-1, MVD = microvessel density, VEGF = vascular endothelial growth factor.

4. Discussion

AQPs were confirmed played an important role in gastric cancer.[16–19] Xu H et al reported AQP4 protein and mRNA expression level in gastric cancer tissue were significantly lower than those in normal gastric tissue.[16] Watanabe T et al found that up-regulation of AQP5 may be involved in differentiation of human gastric cancer cells.[17] Thomas LN et al revealed that high level AQP3, AQP9 and AQP11 mRNA expression were correlated with better OS in gastric patients, whereas AQP0, AQP1, AQP4, AQP5, AQP6, AQP8, and AQP10 mRNA expression were associated with poor OS.[18] Moosavi MS et al found over-expression of AQP1, AQP3, and AQP5 is clearly associated with carcinogenesis, metastasis, reduced survival rate, lymph node metastasis, poorer prognosis, and cellular migration in multiple tumors including gastric cancer.[19]

In the human body, various cells can express VEGF, which plays a key role in promoting the growth of vascular endothelial cells, inducing the formation of new blood vessels and regulating the development of blood vessels, so that it is 1 of the most important vasoactive factors currently known.[20] Furthermore, angiogenesis plays an important role in the growth, invasion and metastasis of cancer cells.[20] Simonetti et al found that the expression of VEGF in tumor tissue was higher than that in normal tissue, suggesting that VEGF can promote tumor microvascular formation and play an important role in the occurrence and development of tumor.[21] In this study, the expression of VEGF in gastric cancer tissues was higher than that in para-cancerous tissues; the expression of MVD in gastric cancer tissues with positive VEGF expression was higher than that in VEGF negative expression tissues. Therefore, it is speculated that VEGF is positively correlated with the expression of MVD in gastric cancer tissues. In addition, VEGF participates in tumor neovascularization, and plays an important role in the formation of cancer and subsequent invasion and metastasis.

MVD can well respond to the state of tumor microangiogenesis, and has been widely used in clinical and basic experiments. MVD is highly expressed in a variety of malignant tumor tissues, but its expression capacity is reduced in normal

Table 4

The correlation between the expression of AQP1, VEGF, and clinicopathological parameters in gastric cancer (n, %).

Clinicopathological parameters	N	AQP1+	χ^2	*P* value	VEGF+	χ^2	*P* value
Tumor size, cm							
≤5	47	36 (76.6)	0.600	0.438	35 (74.5)	0.721	.396
>5	32	22 (68.8)			21 (65.6)		
Gastric cancer differentiation							
High/medium differentiation	35	20 (57.1)	8.529	0.003	15 (42.9)	23.922	<.001
Low/undifferentiated	44	38 (86.4)			41 (93.2)		
Lymph node metastasis							
Yes	51	42 (82.4)	5.886	0.015	42 (82.4)	9.168	.002
No	28	16 (57.1)			14 (50.0)		
Vascular tumor thrombus							
Yes	43	41 (95.4)	23.255	<0.001	41 (95.4)	27.362	<.001
No	36	17 (47.2)			15 (41.7)		
Clinical stage							
I/II	34	15 (44.1)	26.257	<0.001	15 (44.1)	20.724	<.001
III/IV	45	43 (95.6)			41 (91.1)		

AQP1 = aquaporin-1, VEGF = vascular endothelial growth factor.

organs, tissues, and large blood vessels.[22] GRIGORE et al. found that MVD expression in malignant tumors is high, which is closely related to tumor microangiogenesis.[23] The difference between MVD in gastric cancer tissues and para-cancerous tissues in this study was statistically significant, indicating that MVD was closely related to malignant tumors and may be involved in the development of tumors.

In the previous studies of the expression of MVD and AQP1 in gastric cancer, we found that there is little research on the correlation between the MVD and AQP1. But in our study, the expression of MVD in the gastric cancer with positive expression of AQP1 was higher than that in the gastric cancer with negative expression of AQP1, the difference was statistically significant, so it could be predicted that there may be a positive correlation between the expression of AQP1 and MVD in gastric cancer tissues. Both of them were involved in the occurrence and development of gastric cancer, but the relevant data were still insufficient and need further research.

Pan H et al. revealed that there was a positive correlation between AQP1/intratumoral MVD ratio and VEGF expression in endometrial adenocarcinoma.[24] Pulford E et al found AQP1 may interact with VEGF and play a role in vasculogenic mimicry, especially under hypoxic conditions[25] These studies were similar to our results, in gastric cancer tissues AQP1 was positively correlated with the expression of VEGF.

In the process of tumorigenesis and development, a large amount of water and nutrients are needed. The high expression of AQP1 in tumor cells can change the shape, volume and cell epithelial osmotic pressure of the tumor, so as to assist the growth of the tumor and accelerate the infiltration of the tumor into the surrounding tissues.[26] CHANG et al. found that the expression intensity of VEGF and MVD in gastric cancer was related to Borrmann type, tumor differentiation, tumor invasion, lymph node metastasis and TNM stage.[27] The OS of patients with high expression of VEGF and MVD was lower than that of patients with low expression of VEGF and MVD. CARVALHO et al reported that breast cancer patients with high MVD expression had an increased risk of distant metastasis and postoperative recurrence.[28] Other studies have shown that with the increase of MVD, the rate of tumor formation, invasion and metastasis also

Table 5

The correlation between MVD expression and clinicopathological parameters in gastric cancer (X±S).

Clinicopathological parameters	N	MVD	t	*P* value
Tumor size, cm				
≤5	47	42.15±6.03	8.920	.375
>5	32	40.90±6.21		
Gastric cancer differentiation				
High/medium differentiation	35	37.82±4.70	5.980	<.001
Low/undifferentiated	44	44.69±5.35		
Lymph node metastasis				
Yes	51	43.15±5.79	3.111	.003
No	28	38.91±5.78		
Vascular tumor thrombus				
Yes	43	45.87±3.44	10.324	<.001
No	36	36.60±4.53		
Clinical stage				
I/II	34	36.34±4.26	10.303	<.001
III/IV	45	45.62±3.76		

MVD = microvessel density.

increases.[29,30] The results of this study were consistent with the above reports. The expression intensity of AQP1, VEGF and MVD was not related to tumor size, but was closely related to the differentiation, lymph node metastasis, vascular tumor thrombus and clinical stage of gastric cancer. It is inferred that AQP1, VEGF and MVD had a promoting and synergistic effect in the process of tumor formation, invasion and metastasis.

In summary, the expressions of AQP1, VEGF, and MVD in gastric cancer tissues were higher than those in para-cancerous tissues. There was a synergistic effect among the AQP1, VEGF, and MVD, and they were involved in the process of tumor development. Therefore, this study could provide some clinical data for the prognosis evaluation of tumor. In addition, most of the research on anti-tumor drugs was related to new targets. The development of targeted drugs is endless, with a view to launching new highly effective targeted drugs, but it still cannot save majority of patients with advanced tumors, and it is impossible to completely cure tumors. So it still needs further research to explore the mechanism of occurrence and development in gastric cancer, which was aimed to provide new ideas for tumor treatment. Unavoidable, some shortcomings in this study need to state. This study is a retrospective study, and there are inevitably biases. Besides, the size of the study sample is small, large sample was suggested in further research.

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Author contributions

Z. conceived and designed the study, Y. Z. and H. Q. performed the analysis procedures, Y. Z. and H. Q. analyzed the results, H. Q. contributed analysis tools, Y. Z. and H. Q. contributed to the writing of the manuscript. All authors reviewed the manuscript.

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