THE EXPRESSION OF HIF-1α AND GNM IN GASTRIC CANCER AND PARACANCER TISSUES AND THEIR RELATIONSHIP WITH PATIENT PROGNOSIS

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ABSTRACT

Objective: *To investigate the expression of hypoxia-inducible factor-1α (HIF-1α) and glycine methyltransferase (GNM) in gastric cancer and paracancer tissues and their relationship with prognosis.*

Methods: *Fifty patients with gastric cancer who received treatment in our hospital from January 2018 to January 2019 were selected as the research subjects. The expression of HIF-1α and GNMT in gastric cancer and paracancerous tissues was compared, and the relationship between expression and prognosis was analyzed by multivariate Cox regression.*

Results: *The positive expression rate of HIF-1 in gastric cancer tissues was 84.0%, significantly higher than that in paracancer normal tissues (4.0%, P<0.001), and the positive expression rate of HIF-1 in gastric cancer tissues was 64.0%, significantly higher than that in cytoplasm (20.0%, χ^2 = 19.869, P<0.001). The expression of GNMT in gastric cancer tissues was (678.30±221.95) ng/ml, significantly lower than that in paracancer normal tissues (1136.76±332.85) ng/ml (P<0.001). Among the 50 patients, 32 survived, and 18 died. Multivariate logistic regression analysis showed that the expression of HIF-1 protein and GNMT protein, the degree of differentiation of cancer cells, and the depth of infiltration and lymphatic metastasis are independent risk factors affecting the survival and prognosis of patients.*

Conclusion: *HIF-1 is highly expressed in gastric cancer tissues, and GNMT is poorly expressed in gastric cancer tissues. Both markers can be used as important indicators to judge the prognosis of gastric cancer patients.*

Keywords: *Gastric cancer, paracancerous normal tissue, hypoxia-inducible factor 1α, glycine methyltransferase.*

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Introduction

Gastric cancer, caused by malignant tumors of gastric mucosa epithelium, is one of the most common malignant tumors in China, can occur at any age group but mostly in people over 50 years old, and affects male patients twice as often as female patients[(1)]. With the urbanization of society, people's diet is changing, and unhealthy living habits are increasing. While both factors may affect the rate of gastric cancer, the real cause of the disease is still unclear. At present, the main treatment for gastric cancer is surgery. Most patients with early gastric cancer do not have obvious physical symptoms, and only a few patients with early gastric cancer will have pain, vomiting, or similar symptoms suggesting an upper gastrointestinal ulcer disease[(2)].

By the time the patient reports physical pain, weight loss, and other symptoms, gastric cancer has developed into a progressive stage, seriously threatening the life and physical health of patients[(3-4)]. Therefore, exploring effective and specific markers

is very important for the prevention and treatment of early gastric cancer and its prognosis. Studies have found that hypoxia-inducible factor 1α (HIF-1α) is highly expressed in many tumors, which may be closely related to angiogenesis and patient prognosis. Other studies have reported that high HIF-1α expression is associated with prognosis. Glycine methyltransferase (GNMT), with multifunctional proteins, plays an important role in the body.

It is involved in the metabolic process of environmental carcinogens and the process of detoxification. In liver detoxification, GNMT can intercept the chemicals in the cells and then inhibit the production of liver tumors. Some studies have shown that the absence of GNMT expression increases tumor susceptibility, and it also has a role in inhibiting liver cancer[5-6]. This study explored the expression of HIF-1α and GNMT in the gastric cancer group and adjacent normal tissues and the relationship with patient prognosis. The results are reported as follows.

Materials and methods

General information

Fifty patients with gastric cancer who received treatment in our hospital from January 2017 to January 2018 were selected as research objects. Samples of gastric cancer tissues and paracancerous normal tissues of the patients were taken, respectively. Among all the patients, 31 were males, and 19 were females, aged from 30 to 80 years old, with an average age of 58.3±9.3 years old. Nine cases were poorly differentiated, 28 cases were moderately differentiated, and 13 cases were highly differentiated. In terms of TNM staging, 32 cases were in stage I and II, and 18 cases were in stage III and IV. In terms of depth of infiltration (T stage): 28 cases were T1 and T2, and 22 cases were T3 and T4. Twenty-three cases had lymphatic metastasis, and 27 cases had no metastasis.

Criteria for selecting research objects

Inclusion criteria:

• All patients were diagnosed with gastric cancer;

• No radiotherapy or chemotherapy was given before the operation;

• The study was conducted with the consent of the ethics committee of our hospital and the patient himself, and all patients signed the written informed consent.

Exclusion criteria:

• Preoperative chemoradiotherapy;

• Patients who refused to participate in the study.

Treatment plan

Real-time fluorescent quantitative PCR

RNA from all study patients was extracted, and Trizol reagent (Invitrogen, Inc.) was used in strict accordance with its instructions.

• The tissue sample was placed into an EP tube containing 2 ml non-RNA enzyme, and 1ml Trizol reagent was added. After grinding in the liquid nitrogen environment, the sample was left standing at room temperature for 5 min. Then 0.2 ml chloroform was dropped into the tube and shaken for 15 s.

• The sample was centrifuged for 15 min (12000 r/min, 4 °C), the supernatant was separated and added to another tube with 1.5 ml non-RNA enzyme EP, then 0.5 ml of isopropanol was added to the tube. After mixing evenly, the supernatant was allowed to sit at room temperature for 3 min, and then centrifuged at a centrifugation rate of 12000 r/min for 15 min. The supernatant was poured out, and 75% ethanol was added to the tube. The RNA precipitate was washed.

• The above samples were centrifuged for 8 min (12000 r/min, 4 °C), and the supernatant was poured out. After the ethanol was completely evaporated, 50 μl pure water treated with diethyl pyrocarbonate after high-temperature, high-pressure sterilization was added. mRNA was reversed to cDNA using a reversal reagent (Toyobo), and cDNA was then sequenced using a 7900HT sequencer (American Biotechnology) for real-time fluorescent quantitative PCR. Each sample was repeated three times to calculate the relative value.

Western blot test

First, sample proteins were extracted using the RIPA cleavage method, and inhibitors of proteases such as PMSE were added to reduce the degradation rate of proteins. Then, the lysates were centrifuged at a centrifugation rate of 12000 r/min for 10 min (temperature of 4 °C), the supernatant was separated, and the total protein concentration was detected using the BCA kit (from Bianyuanbian).

The protein buffer was added to the lysate, and after a 10 min boiling water bath, the protein was denatured, followed by gel electrophoresis, and transferred to the PVDF membrane.

The skim milk made from TBST (5%

concentration) was used to seal and store the PVDF membrane for 1 h. After the sealing solution was cleaned, the PVDF membrane was incubated in HIF-1 antibody (concentration of 1:1500). The sample was cryopreserved at 4 °C for more than 12 h, then cleaned and incubated with rabbit secondary antibody for 1 h.

Aftercleaning,theenhancedchemiluminescence method was used for shadow development. A reverse transcription-polymerase chain reaction was used to determine the expression of GNMT in cancerous tissues and adjacent normal tissues.

The primers of GNMT were amplified in the PCR instrument, and their relative values were calculated.

Enzyme-linked immunosorbent assay (ELISA)

The GNMT was detected according to the instructions of the ELISA kit.

After adding samples to each pore at 37 °C constant temperature for 2 h, the reagent A was added to continue incubation for 1 h. The reaction solution was sucked out, washed 3 times, incubated with reagent B for 30 min, then washed for 5 times, then incubated with 90 μl of reactant substrate for 20 min and finally mixed with 50 μl of termination solution for absorbance detection.

Statistical methods

All the data were processed and analyzed by SPSS22.0. The number of counting cases [n (%)] was used to represent the count data.

Independent samples between groups were compared using χ^2 tests, the measurement data were represented by mean ± standard deviation ($\bar{x}\pm s$), and the independent sample t-test was used for comparison.

P<0.05 was considered statistically significant. Multivariate Cox regression was used to analyze the prognostic index of gastric cancer patients.

Results

Expression of HIF-1 in gastric cancer tissues and adjacent normal tissues

The positive expression rate of HIF-1 in gastric cancer tissues was 84.0%, significantly higher than that in paracancer normal tissues (4.0%, P<0.001), and the positive expression rate of HIF-1 in gastric cancer tissues was 64.0%, significantly higher than that in cytoplasm (20.0%, 2 = 19.869, P<0.001). See Table 1 for details.

Group	n	Nuclear positive expression	Cytoplasmic positive expression	Total
Gastric cancer tissues	50	32(64.0)	10(20.0)	42(84.0)
Paracancer tissues	50	2(4.0)	0(0)	2(4.0)
χ^2		40.107	11.111	64.935
P		< 0.001	0.001	< 0.001

Table 1: Comparison of HIF-1 cytoplasmic nuclear and cytoplasmic positive rates in gastric cancer and paracancer tissues (n/%).

GNMT expression in gastric cancer tissues and paracancer normal tissues

The expression of GNMT in gastric cancer tissues was 678.30±221.95 ng/ml, significantly lower than that in paracancer normal tissues 1136.76±332.85 ng/ml (P<0.001). See Table 2 for details.

Group	n	Expression of GNMT (ng/ml)
Gastric cancer tissues	50	678.30 ± 221.95
Paracancer tissues	50	1136.76 ± 332.85
T		7.687
P		< 0.001

Table 2: Expression of GNMT in gastric cancer tissues and paracancer normal tissues ($\bar{x}\pm s$).

Univariate analysis of survival and death of gastric cancer patients

Among the 50 patients with gastric cancer, 32 (64.00%) survived, and 18 (36.00%) died.

There was no statistically significant difference in age, gender and other clinical characteristics of the patients with gastric cancer (P>0.05) who died and who lived.

Among them, the degree of differentiation, the depth of tumor invasion, the presence or absence of lymph node metastasis, and the pathological stage were the relevant factors affecting the survival and death of patients (P<0.05). See Table 3 for details.

Influencing patient survival and meta-logistic regression analysis

Analysis results showed that HIF-1 grade, tumor invasion depth, tumor differentiation degree, lymph node metastasis, pathological stage and GNMT were independent risk factors affecting the survival and prognosis of patients. See Table 4 for details.

Factor	N	Survival	Death	χ^2/t	P
Age (year)					
< 40	24	17	7	0.935	0.333
≥ 40	26	15	11		
Gender					
Man	31	23	8	3.679	0.055
Woman	19	9	10		
Degree of differentiation					
Low	9	8	1	9.320	0.002
Middle	28	20	8		
High	13	4	9		
Depth of invasion					
T1~T2	28	22	6	5.864	0.015
T3~T4	22	10	12		
Lymphatic metastasis					
yes	23	10	13	7.785	0.005
no	27	22	5		
Pathological stage					
I~II	32	24	8	4.668	0.031
III~IV	18	8	10		
HIF-1α					
Positive	42	24	18	5.357	0.021
Negative	8	8	0		
GNMT		537.12±89.55	225.77±85.27	12.001	< 0.001

Table 3: Single-factor analysis of survival and death of gastric cancer patients.

Factor	B	Standard error	Wald	df	P	Exp (B)
HIF-1α	18.316	0.312	3450.528	1	< 0.001	1.481E-8
Depth of invasion	–2.120	0.730	8.429	1	0.004	0.120
Degree of differentiation	1.152	0.477	5.833	1	0.016	3.165
Lymphatic metastasis	–1.861	0.726	6.570	1	0.010	0.156
Pathological stage	3.932	1.107	12.615	1	< 0.001	51.000
GNMT	–0.031	0.011	8.411	1	0.004	0.970

Table 4: Results of multivariate logistic regression analysis.

Discussion

The incidence of gastric cancer in digestive system tumors is as high as 40%~50%, which is one of the highest incidences of malignant tumors[7-8]. At present, the main treatment of gastric cancer is surgical treatment, radiotherapy and chemotherapy, traditional Chinese medicine, improving the immunity of patients and other comprehensive treatment[9-10]. Early symptoms of gastric cancer patients are not obvious. By the time patients are diagnosed with gastric cancer, the disease has been present for a long time, and its differentiation, tissue components, biological behavior are very complex. Finding effective specific markers for the identification and treatment of gastric cancer and prognosis has a very important role[11-12] in detecting gastric cancer on the genetic or molecular level and understanding the pathogenesis of gastric cancer. We can effectively improve the rate of early diagnosis of gastric cancer, and then in the early development of treatment, improve the patient prognosis[13-14].

According to the results of this study, the positive expression rate of HIF-1α in the nucleus of gastric cancer tissue was significantly higher than that in cytoplasm. The positive expression rate of HIF-1α in the nucleus and cytoplasm of gastric cancer tissue was significantly higher than that in normal tissue next to the stomach. This finding suggests that the expression of HIF-1α mainly exists in the nucleus and cytoplasm of gastric cancer tissue, and suggests that HIF-1α entering the nucleus may have a certain effect on the vascular endothelial growth factor, regulate the expression of matrix metallase, and affect the metastasis and infiltration of tumor cells.

The results of this study also showed that the positive expression rate of HIF-1α in gastric cancer tissues was significantly higher than that in adjacent normal tissues, while the expression of GNMT in gastric cancer tissues was significantly lower than that in adjacent normal tissues. This finding suggests that HIF-1α and GNMT expression in gastric cancer tissues and adjacent normal tissues are significantly different. In the clinic, gastric cancer can be confirmed and evaluated by detecting the expression of HIF-1α and GNMT in gastric cancer tissues. GNMT is a multifunctional protein that is involved in fat metabolism, cholesterol metabolism in the human body, and in the metabolic process of toxic substances in the liver, so it has the function of detoxification. Detoxification by chemical substance interception might effectively inhibit the production of the tumor[15]. Some studies have reported that the expression of GNMT in gastric cancer tissues is positively correlated with the degree of differentiation and negatively correlated with TNM masses, which suggests that GNMT is related to the progression of gastric cancer[16].

Our study shows that HIF-1α and GNMT are involved in the development of gastric cancer. According to the multiple logistic regression analysis, the depth of tumor invasion, the degree of tumor differentiation, whether lymph node metastases exist, and the pathological stage of the tumor are independent risk factors affecting patient survival. The invasion depth, differentiation degree and pathological stage of the tumor indicate the seriousness of the condition and the disease severity. More serious erosion and tumors in later stages will inevitably affect patient survival and prognosis. Whether or not a tumor has metastasized to the lymph nodes is also an independent risk factor for survival and prognosis.

Conclusion

In summary, HIF-1α is highly expressed in gastric cancer tissues, and GNMT has a low expression level in gastric cancer tissues. Both markers relate to the prognosis of gastric cancer and can be used as an important indicator to predict the prognosis of gastric cancer patients.

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