RESEARCH ARTICLE

Relationship Between Expression of Gastrokine 1 and Clinicopathological Characteristics in Gastric Cancer Patients

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Abstract

The aim of the study was to clarify the role of gastrokine 1 in the process of formation and development of gastric cancer. The expression of gastrokine 1 in gastric cancer and corresponding non-cancerous gastric tissues of 52 gastric cancer patients was assessed with the real-time fluorescence quantitative polymerase chain reaction (RT-PCR) and immunohistochemistry. We also analyzed the relationship between the expression level and clinicopathological characteristics. Gastrokine 1 gene and protein expression in gastric cancer tissues was in both cases significantly lower than in corresponding non-cancerous gastric tissues (both P<0.01), but no significant relationship was found with clinicopathological parameters including tumor location, depth of invasion, differentiation, lymph node metastasis, stage, gender, age and carcinoembryonic antigen (CEA), and carbohydrate antigen 19-9 (CA19-9) level in peripheral blood preoperation of patients (P>0.05, respectively). Furthermore, gastrokine 1 gene expression was markedly lower in gastric cancer tissues of *Helicobacter pylori* (HP)-positive patients than negative ones (P<0.05). The result of the study showed that gastrokine 1 might play a significant role in the process of formation and development of gastric cancer as an anti-oncogene. Its effect might be weakened by HP infection.

Keywords: Gastrokine 1 - gastric cancer - clinicopathology - *Helicobacter pylori*

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Introduction

Gastric cancer, one kind of the most common malignant tumors worldwide, is the second leading cause of cancer death. It was estimated there were about 989 thousand new gastric cancer cases and 738 thousand gastric cancer deaths globally one year (Ferlay et al., 2010). Human gastric mucosa suffers from various wound continually, such as gastric acid, alcohol, too cold or too hot food and so on. Normally because of self repair and protection of gastric mucosa protective factors, injured gastric mucosa can heal soon. However, lessening or lack of protective factors may lead gastric mucosa more exposion to harmful substance, which may induce expression changes of gastric cancer genes, and then promote happening or development of gastric cancer at last. In the past few years one gene named Gastrokine 1, which had the functions such as protecting gastrointestinal mucosa, promoting mucosa repairment, suppressing cancer and so on, was found (Toback et al., 2003). Reducing or lack of its expression plays a significant role in happening and development of gastric cancer (Shiozaki et al., 2001; Rippa et al., 2010). The expression of Gastrokine 1 in gastric cancer and corresponding non-cancerous gastric tissues was detected with RT-PCR and INH. Finally, the relationship between its expression and clinicopathological characteristics of gastric cancer was analysed.

Materials and Methods

Patient characteristics

After inform consent forms were signed, the specimens including gastric cancer and corresponding non-cancerous gastric tissues in general surgery of affiliated hospital of North Sichuan Medical College during April to July in 2011 were collected. All cases were diagnosed as gastric adenocarcinoma with pathological biopsy both preoperation and postoperation. They who had taken anti-HP drug or chemotherapy or radiotherapy lately were removed. The clinicopathological characteristics of

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patients are summarized in Table 1.

Samples

All samples consisted of gastric cancer and corresponding non-cancerous gastric tissues were obtained intraoperation just after they were cut off. Then part were put into -80°C refrigerator quickly used for total RNA extraction. The left were dipped in formalin and then embedded with paraffins for INH.

Detection of HP

Patients were arranged to take ^{14}C-urea breath test to detect HP value preoperation. They whose HP values lower than 100 dpm were regarded as negative, and the others positive. Due to cardia complete obstruction or poor compliance, 11 patients did not accept ^{14}C-urea breath test.

RT-PCR

104 gastric cancer and corresponding non-cancerous gastric tissues were ground into fine powder with mortars and pestles in liquid nitrogen. Then total RNA was extracted with TRizol Reagent (Tiangen, China). Later the quality of total RNA was assessed with ultraviolet spectrophotometer (SHIMADZU, Japan) and agarose gel electrophoresis (agarose from Sigma, America; electrophoresis apparatus from BIO-RAD, America). cDNA was synthesized using reverse transcription kit (BioBRK, China) according to instruction. RT-PCR thermocycler (ABI, America) and kit (Tiangen, China) including Pre Mix, Dye and DNase/RNase free ddH2O were used for cDNA amplification. 1μl cDNA, 10μl Pre Mix, 2μl Dye, forward and reverse primer both 0.6μl (10 pmol/μl) and 5.8μl DNase/RNase free ddH_2O were consisted in amplification system. Gastrokine 1 and β-actin primers were synthesized by invitrogen company. The primer sequence, reaction condition and product size are all in Table 2. All samples were tested duplicately. After amplification finished, dissociation curve was analysed to identify the uniqueness of product. $2^{-\triangle CT}$ was used as relative expression value.

INH

Paraffins embeded 104 gastric cancer and corresponding non-cancerous gastric tissues were sectioned to 3μm in thickness. After the slices were dipped in dimethylbenzene twice each for 10 min, they were put into 100% alcohol, 85% alcohol and 75% alcohol successively, and then washed with running water for deparaffinization. 3% H_2O_2 covered the whole tissues on slices for 20 min at room temperature and away from light in order to block endogenous peroxidase activity. Heat antigen retrieval was performed in 0.01M citrate buffer (ph 6.0) at 95°C for 20 min. Then slices were incubated with mouse anti-Gastrokine 1 monoclonal antibody (ABCAM, England) at a dilution of 1:150 (final concentration: 6.67 μg/ml) at 4°C overnight. At the next moring the slices were incubated with common secondary antibody (Zhongshan Jinqiao, China) at room temperature for 15 min. After colorated with diaminobenzidine and counterstained with hematoxylin, the reaction products were visible. All slices were assessed by two professors of pathology who had no knowledge of any clinicopathological characteristics of the patients. More than 30% epithelial cell cytoplasm stained positively were considered positive (Yoon et al., 2011).

Table 1. Patient Clinicopathological Characteristics

Characteristics		N=52
Gender	Male	41
	Female	11
Age (years)	Mean	58.6±11.7
	Range	31~79
Differentiation	Well	2
	Moderate	14
	Poor	36
Location of tumor	Upper	13
	Middle	14
	Lower	25
Depth of invasion*	T1	10
	T2	10
	T3	0
	T4	32
Lymph node metastasis*	N0	20
	N1	10
	N2	14
	N3	8
Stage*	I	15
	II	10
	III	24
	IV	3

*According to American Joint Committee on Cancer (2010)

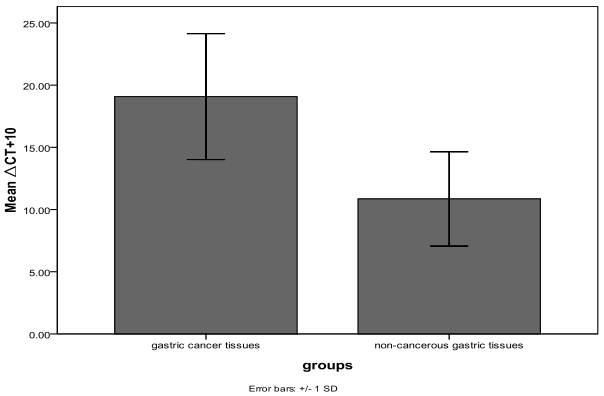

Figure 1. The Mean △CT Value of Gastrokine 1 Gene in Gastric Cancer and Corresponding Non-cancerous Gastric Tissues (Note: The negative numbers of △CT value were too many, so the data in figure 1 were initial data plus 10)

Table 2. Primer Sequence, Reaction Condition and Product Size

Primer name	Primer sequence	Reaction condition	Product size (bp)
Gastrokine 1	F: 5'-AATAACAACGGATGGGACT-3'	95°C 15min	134
	R: 5'-AGGGATTGAATGGAGGG-3'	95°C 10s	
β-actin	F: 5'-GAGCTACGAGCTGCCTGACG-3'	40 cycles	
	R: 5'-GTAGTTTCGTGGATGCCACAG-3'	62°C 30s	120

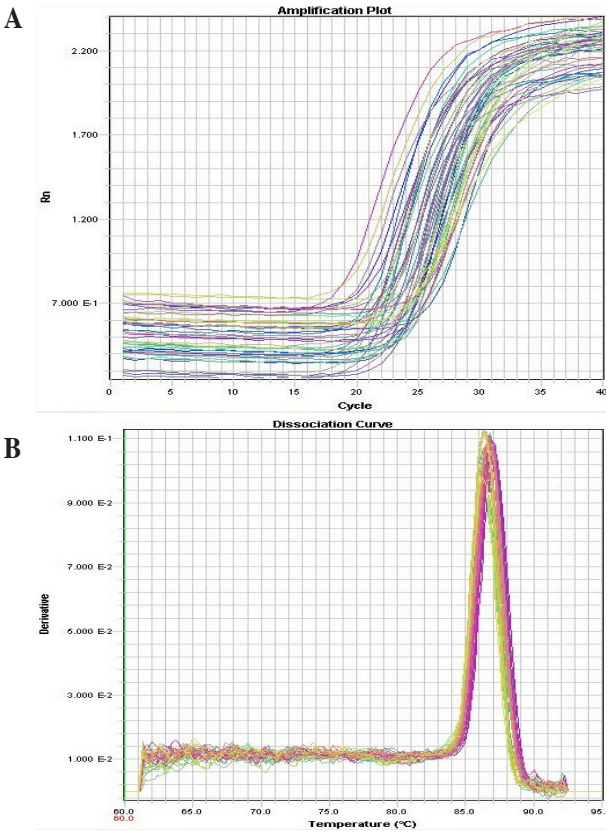

Figure 2. The Amplification Curve (A) and the Dissociation Curve (B)

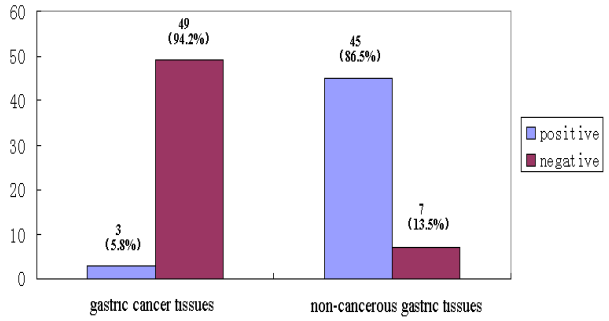

Figure 3. The Expression of Gastrokine 1 Protein in Gastric Cancer and Corresponding Non-cancerous Gastric Tissues

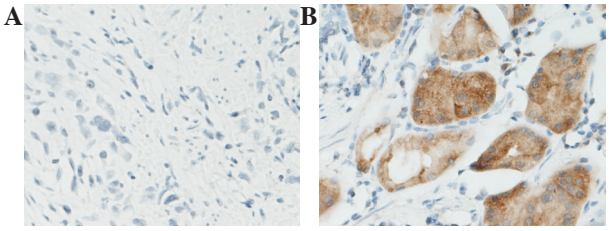

Figure 4. Gastrokine 1 Protein Expression in Gastric Cancer Tissues (Negative) (A) and Corresponding Non-cancerous Gastric Tissues (Positive) (B) (both magnification × 400)

Statistical analysis

T-test or One-way analysis of variance were used for PCR data statistics, and Chi-square test was used for INH results. P<0.05 was considered to have statistical significance.

Table 3. The Expression of Gastrokine 1 Gene in Gastric Cancer Tissues in Different Clinicopathological Characteristics

Characteristics	Number of patients	Expression of Gastrokine 1 gene Mean △CT±SD
Gender		
Male	41	9.53±5.31
Female	11	7.38±3.69
Age (year)		
~49	15	6.52±5.11
50~59	12	10.70±3.07
60~69	15	9.75±5.82
70~	10	9.94±4.94
HP		
+	22	11.14±5.19
-	19	7.67±4.18
Stage		
I	15	9.74±5.29
II	10	8.18±3.61
III~IV	27	9.03±5.49
Lymph node metastasis		
N0	20	8.55±5.35
N1	10	8.83±4.80
N2	14	9.08±5.50
N3	8	10.68±4.38
Differentiation		
Moderate~well	16	9.35±4.75
Poor	36	8.95±5.25
Tumor's location		
Upper	13	9.60±4.58
Middle	14	9.75±5.25
Lower	25	8.42±5.30
Depth of invasion		
T1~T2	20	8.89±4.55
T3~T4	32	9.19±5.42
CEA		
+	6	10.45±3.55
-	43	8.69±5.21
CA19-9		
+	8	8.03±5.04
-	42	9.08±5.03

Results

Expression of Gastrokine 1 gene by RT-PCR

The mean △CT value of Gastrokine 1 in gastric cancer and corresponding non-cancerous gastric tissues were in Figure 1, and the expression variation between them was 8.22±5.01 (t=11.83, P<0.01). The difference was of statistical siginificance. The amplification curve and dissociation curve are in Figure 2.

Relationship between Gastrokine 1 gene expression and HP, clinicopathological characteristics of gastric cancer patients

The mean △CT value of Gastrokine 1 in gastric cancer tissues of HP positive and negative patients were 11.14±5.19 and 7.67±4.18 respectively. The variation was siginificant (t=2.33, P<0.05; Table 3). The CEA data of 3 patients and CA19-9 of 2 patients were lost, so they were removed when made those two statistics analysis. No siginificant relationship was found between Gastrokine 1 and clinicopathological characteristics, including gender, age, tumor's location, depth of invasion, differentiation, lymph node metastasis, stage and CEA, CA19-9 level in peripheral blood preoperation (all P>0.05; Table 3).

Expression of Gastrokine 1 protein by INH

The difference between positive rate of Gastrokine 1 protein in gastric cancer and corresponding non-cancerous gastric tissues, 5.8% (3/52) and 86.5% (45/52), was significant in statistics (P<0.01). See Figure 3 and 4.

Discussion

Gastrokine 1, once named CA11, AMP-18 or foveolin, is located in chromosome 2p13.3. Its full length is about 6 kb, including 6 exon and 5 intron, whose corresponding protein is made up of 185 amino acid. Gastrokine 1 is one part of gastric mucous layer, which is possessed of the functions of promoting gastric mucosa epithelial cells division and migration, maintaining integrity of gastric mucosa, promoting injured mucosa renovation, suppressing neoplasm and so forth (Lacy et al., 1993; Shiozaki et al., 2001; Martin et al., 2003; Toback et al., 2003; Rippa et al., 2010; Yoon et al., 2011). Researches found that from chronic gastritis, atrophic gastritis, gastric mucosa intestinal metaplasia to gastric cancer, the expression of Gastrokine 1 in gastric mucosa reduced gradually (Nardone et al., 2008). So it is thus clear that the expression of Gastrokine 1 changes throughout the gastric canceration, and it plays an important role in the process of formation and development of gastric cancer. If some relationship between Gastrokine 1 expression in gastric cancer tissues and some clinicopathological characteristics can be found, biopsy through gastroscope preoperation may contribute to preliminary assessment of gastric cancer and guide clinical treatment. However, related researches are too few. Whether expression of Gastrokine 1 is related to gastric cancer clinicopathological characteristics and HP or not, past researches can not give a final conclusion. Reports about relationship between expression of Gastrokine 1 and gender, age, CEA and CA19-9 level in peripheral blood preoperation can not be found up to now. Therefore, it is worth lucubration further. In this study, expression of Gastrokine 1 gene and protein in gastric cancer and corresponding non-cancerous tissues were detected using RT-PCR and INH followed with statistics analysis to try to clarify the questions above.

Our experiment results, both Gastrokine 1 gene and protein expressing in gastric cancer tissues were significantly lower than in corresponding non-cancerous tissues, confirmed it is an anti-oncogene (Shiozaki et al., 2001; Rippa et al., 2010; Yoon et al., 2011). Nevertheless, no relationship was found between Gastrokine 1 expression in gastric cancer tissues and pathological characteristics including tumor's location, depth of invasion, differentiation, lymph node metastasis and stage, which conformed to the past two research results (Shiozaki et al., 2001; Yoon et al., 2011). HP infection is related to gastric canceration (Gisbert, 2011). It is an important initiating factor of chronic atrophic gastritis, a precancerous disease of gastric cancer, and can induce the sequential event chronic atrophic gastritis developing to gastric mucosa intestinal metaplasia and to gastric cancer (Ohata et al., 2004; Weck, 2009). Nardon et al. (2008) speculated throught research result that HP infection could down regulate expression of Gastrokine 1, and participate in the early stage of gastric canceration. Our results, Gastrokine 1 expressing notably lower in gastric cancer tissues of HP positive patients than HP negative ones, accorded with their conclusion. In addition, there was no relation between Gastrokine 1 expression and gender, age, CEA and CA19-9 level in peripheral blood preoperation. CEA and CA19-9 level in peripheral blood can be used to assess prognosis of gastric cancer patients (Ucar et al., 2008). So it can be speculated that Gastrokine 1 is unable to act as an indicator of gastric cancer prognosis, but Moss' s research result considered Gastrokine 1 to be related to poorer prognosis of intestinal gastric cancer (Moss et al., 2008). So follow-up to the patients in this study would be very important to make it clear.

Acknowledgements

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