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• GASTRIC CANCER •

Coexpression of cholecystokinin-B/gastrin receptor and gastrin gene in human gastric tissues and gastric cancer cell line

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Abstract

AIM: To compare the expression patterns of cholecystokinin-B (CCK-B)/gastrin receptor genes in matched human gastric carcinoma and adjacent non-neoplastic mucosa of patients with gastric cancer, inflammatory gastric mucosa from patients with gastritis, normal stomachs from 2 autopsied patients and a gastric carcinoma cell line (SGC-7901), and to explore their relationship with progression to malignancy of human gastric carcinomas.

METHODS: RT-PCR and sequencing were employed to detect the mRNA expression levels of CCK-B receptor and gastrin gene in specimens from 30 patients with gastric carcinoma and healthy bordering non-cancerous mucosa, 10 gastritis patients and normal stomachs from 2 autopsied patients as well as SGC-7901. The results were semi-quantified by normalizing it to the mRNA level of β-actin gene using Lab Image software. The sequences were analyzed by BLAST program.

RESULTS: CCK-B receptor transcripts were detected in all of human gastric tissues in this study, including normal, inflammatory and malignant tissues and SGC-7901. However, the expression levels of CCK-B receptor in normal gastric tissues were higher than those in other groups ($P<0.05$), and its expressions did not correlate with the differentiation and metastasis of gastric cancer ($P>0.05$). On the other hand, gastrin mRNA was detected in SGC-7901 and in specimens obtained from gastric cancer patients (22/30) but not in other gastric tissues, and its expression was highly correlated with the metastases of gastric cancer ($P<0.05$).

CONCLUSION: Human gastric carcinomas and gastric cancer cell line SGC-7901 cells coexpress CCK-B receptor and gastrin mRNA. Gastrin/CCK-B receptor autocrine or paracrine pathway may possibly play an important role in the progression of gastric cancer.

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INTRODUCTION

Human beings have developed highly efficient cell-cell communication to integrate and coordinate the proliferation of individual cell types, among which growth factors and hormones play a pivotal role. Gastrointestinal peptides, including gastrin and cholecystokinin (CCK), are a structurally diverse group of molecular messengers that play an important role in the control of appetite and hormonal secretion. Gastrin is secreted by gastrin (G) cells located in the antral part of the stomach, and identified as the circulating hormone responsible for stimulation of acid secretion from the parietal cells. It stimulates gastric enterochromaffin-like (ECL) cells to release histamine, which in turn increases acid secretion via histamine H_2- receptors in parietal cells[1-3]. Recently, it has been demonstrated that gastrin plays a significant role in the proliferation and differentiation of gastric and intestinal epithelial cells[4-6].

Previous studies have shown that hormones and receptors are key molecules in regulating cell growth, differentiation and apoptosis[7]. Cholecystokinin-B (CCK-B)/gastrin receptor belongs to the seven transmembrane G-protein-coupled receptor superfamily and is mainly expressed in parietal cells and ECL cells of gastrointestinal tract. It has been reported that CCK-B receptor on the basolateral cell membrane domain was immunoreactive and showed high-affinity binding sites for gastrin[8]. It has been widely accepted that gastrin, a trophic factor, promotes growth of cancer cells both *in vitro* and *in vivo* through CCK-B receptors, and that expressions of gastrin gene and CCK-B receptor are closely related to the development, progression and invasion of cancer cells, in particular, colorectal and pancreatic cancers[9,10]. Taken together, we propose that gastrin/CCK-B receptors may play an important role in the development and progression of gastric cancers. To test this viewpoint, we detected the levels of gastrin and CCK-B mRNA transcripts in human gastric cancer cell line SGC-7901 and in gastric tissues including gastric cancers and their corresponding normal mucosa tissues, gastritis and normal autopsied stomach specimens.

MATERIALS AND METHODS

Gastric tissues

Thirty gastric cancer specimens (including 12 moderate and 18 low differentiation adenocarcinomas, 22 with and 8 without local lymph node metastases) and surrounding non-tumour mucosa surgically resected from gastric corpus were confirmed histopathologically. Two normal autopsied gastric mucosa specimens were authorized by the Pathology Department of West China Medical School of Sichuan University. Ten gastritis specimens from gastroscopic examination were histopathologically confirmed by Pathology Department of Guiyang Medical College. Tissue samples were immediately stored in RNA protection solution (Omega).

Cell culture

SGC-7901 cells were cultured in RPMI 1640 medium supplemented with 100 mL/L newborn calf serum, 100 units/mL penicillin, and 100 μg/mL streptomycin in a humidified environment of 50 mL/L CO_2 in air at 37 ℃.

Table 1 PCR primers

Gene	Primers	Sequences	Base pairs	GenBank accession number
CCK-B/gas- trin receptor	Sense (545-566)	5'-CGGACTACTCATGGTGCCCTAC-3'	316	L08112.1
	Antisense (861-842)	5'-GCCAACCGCGCCAGTCTCAG–3'		
Gastrin	Sense (166-189)	5'-TAGGTACAGGGGCCAACA-3'	266	NM-000805
	Antisense (431-413)	5'-GGGGACAGGGCTGAAGTG-3'		
β-actin	Sense (320-340)	5'-TGGAGAAAATCTGGCACCAC–3'	190	BC016045
	Antisense (509-489)	5'-GAGGCGTACAGGGATAGCAC-3'		

CCK-B: cholecystokinin-B.

Reverse transcription

Total RNA was extracted from gastric samples or 10^6 cells using TRIzol (Invitrogen) reagent according to the manufacturer' s recommendations. Five microgramme of total RNA was used as a template for the first-strand cDNA synthesis when the reaction mixture consisted of 120 units of MMLV (Maloney murine leukemia virus) reverse transcriptase, 5 μmol/L random hexamer oligonucleotide primer, 10 mmol/L dithioerythritol, 2 mmol/L dNTP, and 1×first-strand buffer in a total volume of 20 μL. RNA was denatured at 65 ℃ for 15 min and immediately chilled on ice, and then incubated for 10 min at room temperature before reverse transcriptase was added. The reverse transcription was performed for 60 min at 37 ℃ and terminated by heating to 95 ℃ for 10 min.

PCR analysis of CCK-B receptor and gastrin

The primers used for PCR amplification in the study are listed in Table 1. Two microliter of reverse transcripts was amplified by PCR in a total volume of 50 μL containing 0.1 μmol/L oligonucleotide primers for CCK-B receptor or gastrin and β-actin, respectively, 250 μmol/L dNTP, 2 mmol/L $MgCl_2$, and 1×PCR buffer. cDNAs were denatured for 5 min at 95 ℃ before 2.5 units *Taq* DNA polymerase was added. The conditions of touch-down PCR were at 94 ℃ for 45 s, at 68-62 ℃ for 1 min for CCK-B receptor or at 60-55 ℃ for 1 min for gastrin with decreasing 1 ℃ per cycle at beginning, and at 72 ℃ for 1 min for 35 cycles. PCR products were visualized by agarose gel electrophoresis and ethidium bromide staining.

Semi-quantitative analysis of CCK-B/gastrin receptor gene expression

PCR was performed simultaneously by adding the specific primers for both CCK-B/gastrin receptor and β-actin in a single reaction system after reverse transcription. PCR products were separated by 15 g/L agarose gel and the results of electrophoresis were photographed. The level of CCK-B/ gastrin receptor mRNA expression *vs* β-actin was semi-quantified by Lab image software and the data were expressed as mean±SD and followed statistical analysis through one-way ANOVA, Student-Newman-Keuls multiple comparisons and independent-samples *t* test by SPSS 10.0. Statistical significance was assumed when $P<0.05$.

Sequencing

The PCR products of CCK-B/gastrin receptor amplified from the gastric tissues were separately purified by a gel extraction kit, and sequenced by an ABI sequencing machine. The sequences were compared with the GenBank database using BLAST analysis.

RESULTS

Expression of CCK-B receptor gene in human gastric tissues and SGC-7901 cells

CCK-B receptor expression was indicated by the presence of a 316-bp PCR product, and the gastrin gene expression yielded a 266-bp PCR product while the β-actin mRNA as an internal control revealed a 190-bp product on agarose gel. Figure 1 shows the expression of CCK-B/gastrin receptor detected in all specimens taken from normal, inflammatory, cancerous gastric tissues and SGC-7901 cells.

Then, all of the samples were further divided into three groups including normal (surrounding healthy and autopsied gastric tissues, $n=32$), inflammatory ($n=10$) and malignant groups ($n=30$) which were subdivided into the local lymph node metastases ($n=22$) and non-metastases ($n=8$) groups, or moderate (12) and low (18) differentiation adenocarcimoma groups. Data were analyzed by one-way ANOVA. As shown in Figure 2, the expression level of CCK-B receptor mRNA in surrounding healthy gastric tissues is significantly higher than that in neoplastic and inflammatory tissues ($P<0.05$) while there are neither significant differences between metastases and non-metastases groups nor between groups with different differentiations ($P>0.05$).

Gastrin gene expression in human gastric cancer tissues and SGC-7901 cells

Gastrin mRNA was detected on both SGC-7901 cells and gastric cancer specimens (22/30), among which 86.4%(19/22) with local lymph node metastases, and 10.0%(3/30) without metastases. The positive expression rate of gastrin mRNA in metastatic cases was significantly higher than that in lymph node metastasis-negative cases ($P<0.05$). Gastrin mRNA was detected in 9 out of 12 gastric adenocarcinoma specimens with moderate differentiation and in 13 out of 18 cancerous specimens with poor differentiation. Statistical analyses showed no significant difference between the poor and moderate differentiation groups ($P>0.05$). Surprisingly, gastrin mRNA transcripts were detected neither in normal gastric tissues nor in inflammatory ones (Figure 1).

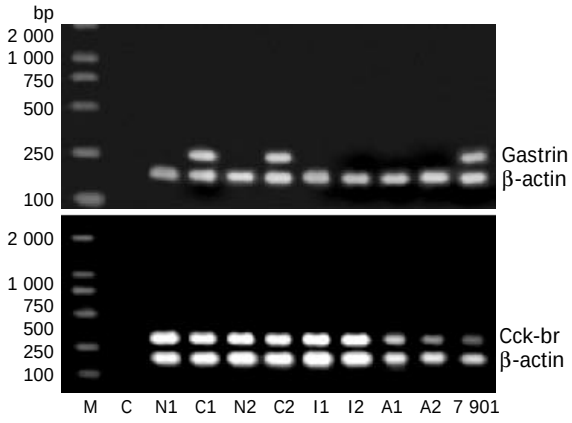

Figure 1 Expression of CCK-B receptor (below) and gastrin (above) mRNA in human gastric tissues and gastric cancer cell line. Total RNA extracted from matched tumor (C1, C2) and non-neoplastic (N1, N2) gastric tissues of patients with gastric

cancer, inflammatory gastric mucosa (I1 and I2) from patients with gastritis, normal stomachs from two autopsied patients (A1, A2) and SGC-7901 cells (7901) were analyzed by RT-PCR. The second lane (C) corresponds to the negative control (H_2O). The results are representatives of all specimens. Cck-br and gastrin stand for cholecystokinin B receptor and gastrin gene, respectively. β-actin: β-actin gene. M: marker (bp).

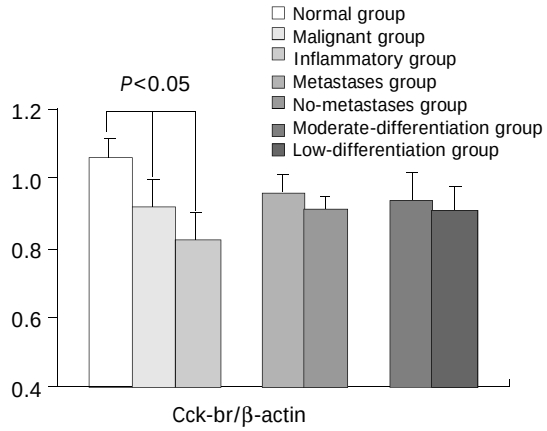

Figure 2 Semi-quantitative analysis of CCK-B receptor expression in human gastric tissues. The expression levels of CCK-B receptors were higher in normal group than in malignant and inflammatory groups, but there was no significant difference compared with other groups. Cck-br/β-actin: ratio of the intensities of the bands of CCK-B receptor *vs* β-actin on agarose gel. The results are expressed as mean±SD in every group.

Confirmation of CCK-B receptor and gastrin genes from samples

The gene sequence identities of CCK-B receptor/gastrin amplified from four different gastric tissues were 100% and 96%, respectively, as compared with CCK-B receptor/gastrin gene sequences from GenBank (Figure 3).

DISCUSSION

CCK-B receptor is widely distributed throughout the human gastrointestinal tract, pancreas, lung and some neuroendocrine tissues. It mediates the normal physiological function of gastrin. Gastrin has proliferative effects on various malignancies including gastric, colorectal, pancreatic, medullary thyroid cancers, small cell lung cancer as well as tumors of the central and peripheral nervous systems through CCK-B receptors[11-17].

Coexpression of gastrin gene and CCK-B receptor in cancer tissues *in vivo* or cell lines *in vitro* may suggest the existence of autocrine or paracrine pathways, through which gastrin exerts its physiological or pathological effects. All of the gastric specimens tested expressed CCK-B receptor mRNA, but only gastric cancer samples (22/30) expressed gastrin transcript and the expression levels of gastrin mRNA were closely correlated with the progression and metastasis of malignant tumors. However, there was no correlation between the expression level of gastrin mRNA and the grade of tumor differentiation. Taken together, our results suggested that the existence of an autocrine or paracrine pathway of gastrin/CCK-B receptors might play an important role in the pathogenesis, especially in the progression, of gastric carcinomas.

SGC-7901 cells were originated from metastatic lymph nodes of a Chinese patient with gastric cancer in 1981. Coexpression of CCK-B receptor and gastrin gene in this cell line provided further evidences that the gastrin/CCK-B autocrine loop might be involved in the development of human gastric cancer. This autocrine loop has also been proved to be functional in human colorectal[9], pancreatic[18] and lung[19,20] carcinomas.

Using semi-quantitive method, we demonstrated that the expression levels of CCK-B receptor mRNA in normal gastric tissues were higher than those in gastric cancer and gastritis, and its expression did not correlate with the grade of differentiation and metastases of gastric cancers. Henwood's group[21] reported that a significantly increased expression of CCK-B receptor protein was seen along the pathway from normal gastric tissues, gastritis to gastric adenocarcinoma by immunohistochemistry. Other malignant tumors also showed the inconsistency with a published paper[22].

Recent insights into CCK receptors have improved our understanding of new receptor isoforms including CCK-C receptor, intron 4-containing splice variant of CCK-B receptor and glycine-extended gastrin receptor besides classic CCK-A and CCK-B receptor subtypes[23-25]. These new receptor subtypes have been found to present in many human malignancies and cell lines and to have a functional role in mediating gastrin's proliferative effects on malignancies[26,27].

Apart from CCK receptor heterogeneity, post-translational processing of pro-gastrin is known to increase glycine-extended gastrin and progastrin other than the amidated forms known to be biologically active. Although extended forms have limited biological activities in the stimulation of gastric acid secretion, it has been found that they exist in many cancers and cultured cell lines and have amidated gastrin-independent trophic activities[28-32]. Unfortunately, to date, no specific receptors have been characterized and no specific antagonists are available, making it difficult to study their potential effects on the pathogenesis of carcinomas.

In conclusion, the coexpression of gastrin gene and CCK-B receptors in SGC-7901 cells and gastric carcinoma tissue suggests that a functional autocrine loop exists and may play an important role in the pathogenesis and progression of human

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Cck-br      92   tactgctgcttctgctcttgttcttcatcccgggtgtggttatggccgtg   141
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||
L08112.1   659   tactgctgcttctgctcttgttcttcatcccgggtgtggttatggccgtg   708

Gastrin    138   agaagaagaagcctatggcatggatggacttcggccgccgcagtgctgag   187
                 ||||||||||||||||||||||||||||||||||||||||||||||||||||
NM_000805  312   agaagaagaagcctatgg-atggatggacttcggccgccgcagtgctgag   359
```

Figure 3 Sequence analyses of CCK-B receptor/gastrin mRNA from samples. L08112.1: Human brain CCK-B/gastrin receptor gene from GenBank. NM_000805: Homo sapiens gastrin gene from GenBank. Cck-br and gastrin: CCK-B receptor and gastrin gene sequences from specimens, respectively. The results showed are representatives of four samples.

gastric carcinomas. However, human tumor cells can express different CCK-B receptor subtypes and different bioactive forms of gastrin amidated and non-amidated, and that complex networks may exist among these components. More studies are needed for their roles in the development of human gastric carcinomas.

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