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COLORECTAL CANCER

Relationship between expression of gastrin, somatostatin, Fas/FasL and caspases in large intestinal carcinoma

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Abstract

AIM: To explore the correlation between the mRNAs and protein expression of gastrin (GAS), somatostatin (SS) and apoptosis index (AI), apoptosis regulation gene Fas/FasL and caspases in large intestinal carcinoma (LIC).

METHODS: Expression of GAS and SS mRNAs were detected by nested RT-PCR in 79 cases of LIC. Cell apoptosis was detected by molecular biology *in situ* apoptosis detecting methods (TUNEL). Immunohistochemical staining for GAS, SS, Fas/FasL, caspase-3 and caspase-8 was performed according to the standard streptavidin-biotin-peroxidase (S-P) method.

RESULTS: There was a significant positive correlation between mRNA and protein expression of GAS and SS ($^{GAS}r_s$=0.99, $P < 0.01$; $^{SS}r_s$ = 0.98, $P < 0.01$). There was significant difference in positive expression rates of GAS, SS mRNAs and protein among different histological differentiation, histological types and Dukes' stage of LIC. The AI in GAS high and moderate expression groups was significantly lower than that in low expression groups (3.75 ± 2.38 *vs* 7.82 ± 2.38, $P < 0.01$; 5.51 ± 2.66 *vs* 7.82 ± 2.38, $P < 0.01$), and the AI in SS high and moderate expression groups was significantly higher than that in low expression groups (9.03 ± 1.76 *vs* 5.35 ± 3.00, $P < 0.01$; 7.44 ± 2.67 *vs* 5.35 ± 3.00, $P < 0.01$). There was a significant negative correlation between the integral ratio of GAS to SS and the AI (r_s = -0.41, $P < 0.01$). The positive expression rate of FasL in GAS high and moderate expression groups was higher than that in low expression group (90.9% and 81.0% *vs* 53.2%, $P < 0.05$). The positive expression rates of Fas, caspase-8 and caspase-3 in SS high (90.0%, 90.0% and 100%) and moderate (80.0%, 70.0%, 75.0%) expression groups were higher than that in low expression group (53.1%, 42.9%, 49.0%) (90.0% and 80.0% *vs* 53.1%, $P < 0.05$; 90.0% and 70.0% *vs* 42.9%, $P < 0.05$; 100.0% and 75.0% *vs* 49.0%, $P < 0.05$). There was a significant positive correlation between the integral ratio of GAS to SS and the semiquantitative integral of FasL (r_s = 0.32, $P < 0.01$).

CONCLUSION: GAS and SS play important roles in the regulation and control of cell apoptosis in LIC, and the mechanism may be directly related to the aberrant expression of Fas/FasL. The GAS and SS will be valuable targets of the biological behavior of LIC.

Key words: Large intestinal carcinoma; Gastrin; Somatostatin; Apoptosis index; Fas; FasL; Caspase

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INTRODUCTION

Large intestinal cancer (LIC) is one of the most common digestive tract malignant tumors in the world, with a high incidence rate in North America, Western Europe, Australia, New Zealand and France, and is the second leading cause of gastrointestinal cancer-related mortality worldwide[1-4]. In China, it now ranks fourth[5]. Although great progress in understanding the molecular aspects of LIC has been made and several therapeutic agents have been developed, it is still difficult to cure the tumor. It is posing a serious threat to public health and remains a major killer among Chinese patients. The general survival rate

of LIC patients does not exceed 40%. Thereby, it is very important to know what kind of cell factor can influence cell apoptosis, which will enrich the etiology theory of tumor. In addition, while studying the mechanism of loss of control of tumor cell apoptosis from signal transduction pathway, we may find a new way to treat malignant tumors. Previous studies have demonstrated that the occurrence of LIC is directly related to the abnormal expression of gastrointestinal hormones such as gastrin (GAS), somatostatin (SS), *etc*[6,7]. Some studies found that GAS was able to promote growth and inhibit apoptosis of LIC cells, but the role of SS was opposite[8-10]. However, the detailed molecule mechanism by which GAS and SS mediate cell apoptosis of LIC is not fully known. We used nested RT-PCR method to detect the expression of GAS and SS mRNAs in LIC tissues, and TUNEL method to detect cell apoptosis, and immunohistochemical staining S-P method to detect the protein expression of GAS, SS, Fas/FasL, caspase-3 and caspase-8. The aim of this study was to explore the correlation between the mRNAs and protein expression of GAS and SS, and between the protein expression of GAS, SS and apoptosis index (AI), and apoptosis regulation gene Fas/FasL and caspases in LIC, and to further confirm whether GAS and SS could mediate LIC cell apoptosis mainly via affecting the expression of Fas/FasL.

MATERIALS AND METHODS

Clinical data

Seventy-nine cases of cancer tissue samples were randomly and retrospectively selected from patients with LIC hospitalized in our hospital from January 2004 to October 2006. All patients were confirmed as having LIC by clinical pathology. Among them, 36 were cases of rectum cancer, and 43 were cases of colorectal carcinoma. Thirty-seven were females and 42 were males. The median age was 50.8 ± 11.2 years, with a range of 21-79 years. There were 27 patients with well differentiated carcinoma, 28 patients with moderately differentiated carcinoma, and 24 patients with poorly differentiated carcinoma (inclusive of undifferentiated carcinoma). Among them, there were 40 cases of ulcerative type, 32 protruded type, 7 infiltrating type, 19 papillary adenocarcinoma, 35 glandular adenocarcinoma, 10 mucoid carcinoma, 6 signet-ring cell carcinoma and 9 undifferentiated carcinoma. The clinical stage was determined according to the Dukes' stage. Dukes' stages A and B were found in 36 patients, and stages C and D in 43 patients.

Main reagents

The polyclonal rabbit antibodies against human SS protein and human GAS, monoclonal rabbit antibodies against human Fas and FasL, caspase-3, caspase-8, and immunohistochemical staining kit and *in situ* cell apoptosis detection kit Ⅱ were all purchased from Beijing Zhongshan Biological Technology Co, Ltd. Trizol liquid, M-MLV reverse transcriptase, SK312, Taq DNA polymerase, RNAsin, oligo (dt) 15 and dNTPs primers were purchased from Shanghai Sangon Biological Engineering Technology and Service Co. Ltd and PCR primers were synthesized by the same company.

Total RNA extraction and cDNA synthesis

Total RNA was extracted from frozen tissue specimens (50-100 mg) using TRIzol reagent according to the protocol provided by the manufacturer. Total RNA was primed with an Oligo (dT) 15 oligonucleotide and reverse-transcribed following the manufacturer's instructions.

PCR amplification

The primers of GAS, SS and β-actin were synthesized according to the primer design principles, all primers spanned an intron to control against amplification of genomic DNA sequences. Two point five μL first strand cDNA was amplified in 25 μL total volume. The first PCR amplifications were performed in a UNO Ⅱ thermocycler (Perkin-Elmer, USA) under the following conditions. After an initial denaturation for 5 min at 94℃, samples were subjected to 30 cycles, each amplification consisting of denaturation at 94℃ for 30 s, primer annealing at 60℃ for 45 s, extension at 72℃ for 45 s, followed by a final extension of 6 min 72℃. Conditions and parameters of the second PCR amplifications were the same with the first PCR amplifications except for primers. The length of final PCR products was 174 base pair (bp) (GAS) and 231 bp (SS), respectively. At last, 6 μL of reaction product was analyzed by electrophoresis on a 1.5% agarose gel followed by ethidium bromide staining and digital camera photography (Table 1, Figure 1).

Detection of cell apoptosis

Cell apoptosis was detected using molecular biology *in situ* apoptosis detecting methods (TUNEL). The sections were fixed and embedded conventionally. Dnase (1 mg/L) was used to digest the positive control specimen for 10 min before put into TUNEL response mixture. Only label liquor was added into the negative control response mixture. The detailed manipulation was conducted according to the introductions for users. Cells with brown or yellow nuclei were assumed as apoptotic cells. The number of apoptotic cells and total cancer cells was counted under light microscope at 400 × magnification in 10 fields of vision and the average values were obtained for the calculation of apoptosis index (AI) according to the formula: AI = (apoptotic cells/total cancer cells) × 100%.

Immunohistochemical staining

Specimens obtained at surgery were routinely fixed in 10% neutral formalin and embedded in paraffin. Serial 4 μm thick sections were cut. Immunohistochemical staining for Fas, FasL, caspase-3, caspase-8, GAS and SS was performed by the standard streptavidin-biotin-peroxidase (S-P) method. Positive pancreatic tissue and stomach antrum mucous membrane were used as a positive control for SS and GAS. Positive controls for Fas, FasL, caspase-3 and caspase-8 were purchased from Shanghai Sangon Biological Engineering Technology and Service Co. Ltd. PBS 0.01 mol/L as a negative control replaced the primary antibody. The detailed

Table 1 Primers used for nested RT-PCR amplification of GAS and SS

Name	Primer sequence	PCR conditions	Size (bp)
GAS	1: 5′-TATGTGCTGATCTTTGCACTGGCT-3′ (sense: 6307-6330)	94℃, 30 s	282
	2: 5′-CTCATCCTCAGCACTGCGGCGGCC-3′ (antisense: 6718-6695)	60℃, 45 s	
		72℃, 45 s	
GAS	3: 5′-GAGCTACCCTGGCTGGAGCAGCAG-3′ (sense: 6415-6438)	94℃, 30 s	174
	2: 5′-CTCATCCTCAGCACTGCGGCGGCC-3′ (antisense: 6718-6695)	60℃, 45 s	
		72℃, 45 s	
SS	1: 5′-ATGCTGTCCTGCCGCCTCCAG-3′ (sense: 106-126)	94℃, 30 s	348
	2: 5′-ACAGGATGTGAAAGTCTTCCA-3′ (antisense: 1330-1310)	60℃, 45 s	
SS	3: 5′-GCTGCTGCCGCGGGGAAGCAG-3′ (sense: 223-243)	94℃, 30 s	231
	2: 5′-ACAGGATGTGAAAGTCTTCCA-3′ (antisense: 1330-1310)	60℃, 45 s	
		72℃, 45 s	

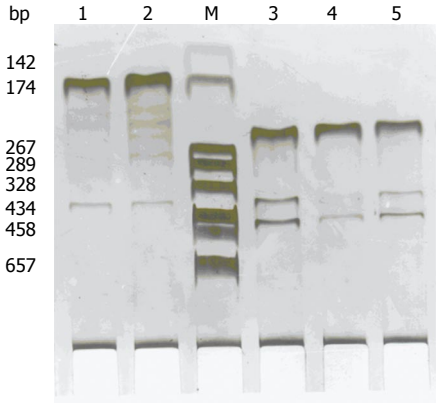

Figure 1 Expression of GAS, SS mRNA by nested RT-PCR analysis in LIC tissue. Lane M: DNA marker DL 2000; Lane 1-2: Positive expression of GAS; Lane 3-5: Positive expression of SS.

manipulation of S-P immunohistochemical method was used according to its manual.

Evaluation of scores

Brown-yellow staining mainly in cell plasma, partly in cell membranes was considered positive SS and GAS protein expression. When SS and GAS protein expression was scored, both the extent and intensity of immunopositivity were considered. The intensity of staining was scored as follows: 0, no staining; 1, weak-yellow; 2, brown-yellow; and 3, brown-black. The extent of positive cells was scored as follows (100 cells were counted by two independent observers, who did not know the clinicopathological features of these LIC): 1, positive staining cells < 5%; 2, positive staining cells in 5%-10%; 3, positive staining cells in 10%-20%; and 4, positive staining cells > 20%. The final score was determined by multiplying the intensity and extent of positivity scores, yielding a range from 1 to 12. According to the semi-quantitative integral evaluation, SS and GAS were divided into three groups: Scores 1-3 was defined as the low expression group, 4-6 as the moderate expression group, and 7-12 as the high expression group (Figure 2A and B).

The positive Fas and FasL protein expression was defined with brown-yellow staining mainly in cell membranes, or cell plasma, while positive caspase-3 and caspase-8 protein expression was defined with brown-yellow staining mainly in cell plasma. The degree of Fas, FasL, caspase-3 and caspase-8 staining was estimated by semi-quantitative evaluation and categorized by the extent and intensity of staining[11]. The intensity of staining was scored as: 0, negative; 1, weak-yellow; 2, brown-yellow; and 3, brown-black. The extent of positive cells was scored as: 0 = positive staining cells in 0%-5%, 1 = positive staining cells in 6%-25%, 2 = positive staining cells in 26%-50%, 3 = positive staining cells in 51%-75%, and 4 = positive staining cells > 75%. Combined staining score was used to evaluate the results of Fas, FasL, caspase-3 and caspase-8 staining. The final score was determined by adding the intensity and extent of staining scores, yielding a range from 0 to 7. Scores 1-2 were defined as negative expression (-), 3 as weak staining (+), 4 as moderately staining (++), and ≥ 5 as strong staining (+++) (Figure 2C-F).

Statistical analysis

Statistical evaluation was performed using Chi-square test and q test to differentiate the positive rates of different groups, and using Spearman test to analyze the correlation between the ratio of GAS to SS and the integral of Fas, FasL, caspase-3 and caspase-8. All data were analyzed with statistical package for social science (SPSS) version 10.0. $P < 0.05$ was considered statistically significant.

RESULTS

Expression of GAS, SS mRNA and protein

The positive expression rates of GAS and SS mRNA were 46.8% (36/79), 41.8% (33/79), respectively. The positive expression rates of GAS and SS protein were 40.5% (32/79) and 38.0% (30/79), respectively. There was a significant positive correlation between mRNA and protein expression of GAS and SS ($P < 0.01$, ${}^{GAS}r_s = 0.99$; $P < 0.01$, ${}^{SS}r_s = 0.98$). There was significant difference in positive expression rates of GAS, SS mRNAs and protein among different histological differentiation, histological types and Dukes' stage of LIC, as shown in Table 2.

Apoptosis index

The AI in GAS high and moderate expression groups was significantly lower than in low expression groups ($q_{high\ vs\ low} = 6.71$, $P < 0.01$; $q_{middle\ vs\ low} = 4.60$, $P < 0.01$), and the AI in SS high and moderate expression groups was significantly higher than in low expression groups ($q_{high\ vs\ low} = 5.66$, $P < 0.01$; $q_{middle\ vs\ low} = 4.21$, $P < 0.01$). There was a significant negative correlation between the integral ratio of GAS to SS and the AI ($r_s = -0.41$, $P < 0.01$) (Table 3).

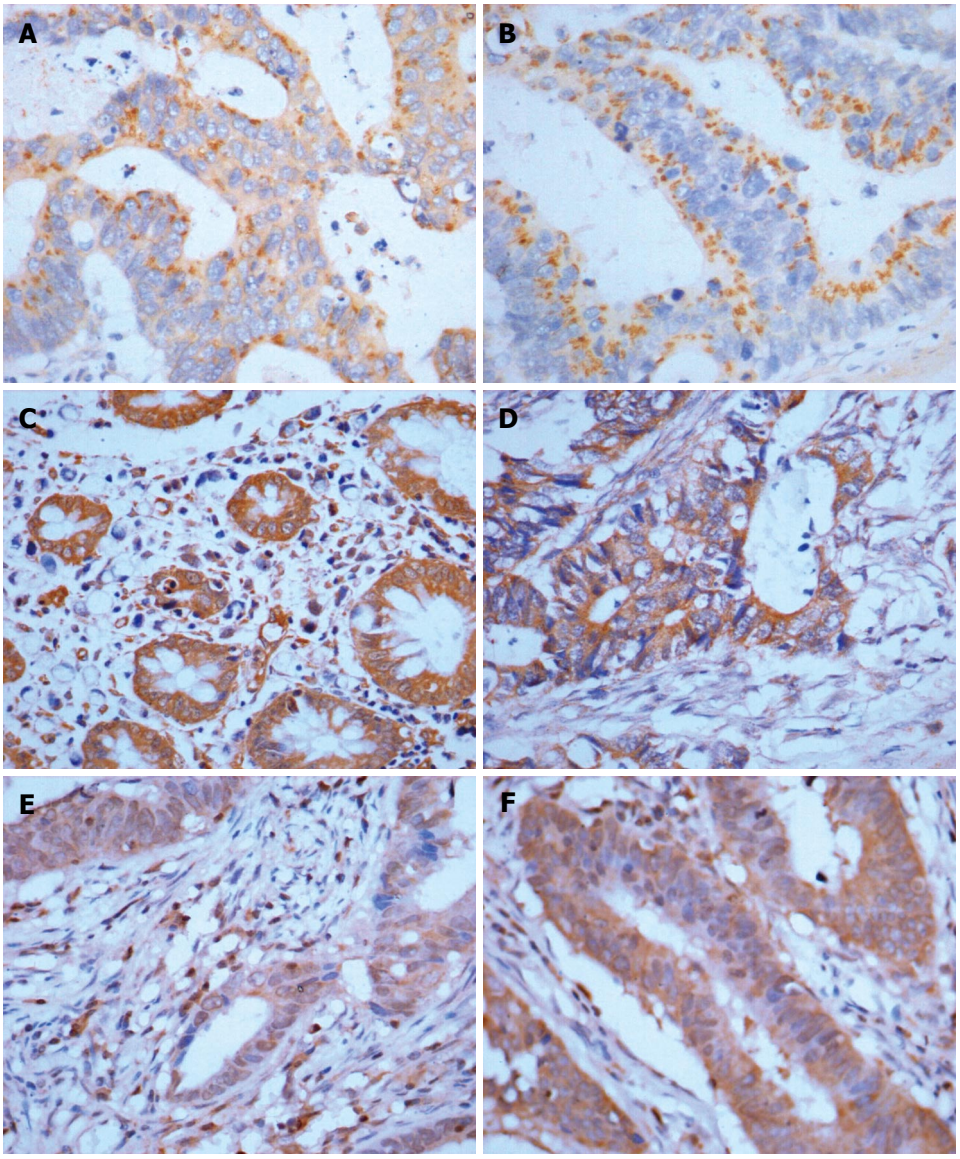

Figure 2 Strong expressions of GAS, SS, Fas, FasL, caspase-3 and caspase-8 in LIC tissues. **A**: Strong GAS expression in LIC. S-P × 400; **B**: Strong SS expression in LIC. S-P × 400; **C**: Strong Fas expression in SS high expression group. S-P × 400; **D**: Strong FasL expression in GAS high expression group of colorectal carcinoma. S-P × 400; **E**: Strong caspase-3 expression in SS high expression group. S-P × 400; **F**: Strong caspase-8 expression in SS high expression group. S-P × 400.

Table 2 Correlation between clinicopathological factors and GAS and SS mRNA and protein expression in LIC

Groups	*n*	mRNA						Protein					
		GAS (+)	χ^2	*P*	SS (+)	χ^2	*P*	GAS (+)	χ^2	*P*	SS (+)	χ^2	*P*
Differentiation													
Well	27	8[b]	10.47	< 0.01	18			6[b]	10.23	< 0.01	17	5.24	< 0.05
Moderate	28	11[a]	6.68	< 0.05	10[c]		< 0.05	10[a]	4.95	< 0.05	9[c]	11.24	< 0.01
Poor	24	18			5[d]	10.78	< 0.01	16			4[d]		
Histological types													
Papillary	19	6[e]	4.80	< 0.05	13			4[e]	6.22	< 0.05	12		
Tubular	35	13[e]	4.40	< 0.05	14			11[e]	4.38	< 0.05	14		
Mucinous and signet-ring	16	11			4[g]	6.56	< 0.05	10			3[g]	6.99	< 0.05
Undifferentiated	9	7			2[g]	5.44	< 0.05	7			1[g]	7.33	<0.05
Dukes' stage													
A and B	36	12[i]	4.84	< 0.05	20[i]	5.17	< 0.05	10[i]	4.44	< 0.05	18[i]	4.06	< 0.05
C and D	43	25			13			22			12		

[a]$P < 0.05$, [b]$P < 0.01$ *vs* poor differentiation; [c]$P < 0.05$, [d]$P < 0.01$ *vs* well differentiation; [e]$P < 0.05$ *vs* mucinous and signet-ring and undifferentiated; [g]$P < 0.05$ *vs* papillary; [i]$P < 0.05$ *vs* Dukes' stages C and D.

Fas, FasL, caspase-3 and caspase-8 expression in GAS and SS high, moderate and low expression groups of LIC

The positive expression rate of FasL in GAS high (10/11) and moderate (17/21) expression groups was higher than that in low expression group (26/47) ($\chi^2_{\text{high } vs \text{ low}} = 6.24$, $P < 0.05$; $\chi^2_{\text{moderate } vs \text{ low}} = 4.74$, $P < 0.05$). There were no significant differences in positive expression rates of Fas, caspase-3 and caspase-8 among GAS high, moderate and

Table 3 Comparison of AI in GAS and SS high, moderate and low expression groups of LIC (mean ± SD)

Groups	*n*	A I (mean ± SD)	*F*	*P*
SS				
Low	10	5.35 ± 3.00		
Moderate	20	7.44 ± 2.67[b]	17.63	< 0.01
High	49	9.03 ± 1.76[b]		
GAS				
Low	47	7.82 ± 2.38		
Moderate	21	5.51 ± 2.66[d]	16.08	< 0.01
High	11	3.75 ± 2.38[d]		

[b]$P < 0.01$ *vs* low SS group; [d]$P < 0.01$ *vs* low GAS group.

Table 4 Comparison of positive rates of Fas, FasL, caspase-3 and caspase-8 protein in GAS and SS high, moderate and low expression groups of LIC

Groups	*n*	Fas		FasL		Caspase-3		Caspase-8	
		+ (%)	-	+ (%)	-	+ (%)	-	+ (%)	-
GAS									
High	11	7 (63.6)	4	10 (90.9)[a]	1	9 (81.8)	2	8 (72.7)	3
Moderate	21	16 (76.2)	5	17 (81.0)[a]	4	14 (66.7)	7	13 (61.9)	8
Low	47	37 (78.7)	10	26 (53.2)	21	29 (61.7)	18	28 (59.6)	19
SS									
High	10	9 (90.0)[c]	1	8 (80.0)	2	10 (100.0)[c]	0	9 (90.0)[c]	1
Moderate	20	16 (80.0)[c]	4	14 (70.0)	6	15 (75.0)[c]	5	14 (70.0)[c]	6
Low	49	26 (53.1)	23	32 (65.3)	17	24 (49.0)	25	21 (42.9)	28

[a]$P < 0.05$ *vs* GAS low expression group; [c]$P < 0.05$ *vs* SS low expression group.

low expression groups ($P > 0.05$). The positive expression rates of Fas, caspase-8 and caspase-3 in SS high (9/10, 9/10 and 10/10) and moderate (16/20, 14/20 and 15/20) expression groups was higher than that in low expression group (26/49, 21/49 and 24/49) (χ^2_{high} *vs* low = 5.48, 5.62 and 6.89, $P < 0.05$; χ^2_{middle} *vs* low = 4.32, 4.19 and 3.91, $P < 0.05$). There was no significant difference in positive expression rate of FasL among SS high, moderate and low expression groups ($P > 0.05$) (Table 4). There was a significant positive correlation between the integral ratio of GAS to SS and the semi-quantitative integral of FasL ($r_s = 0.32$, $P < 0.01$). But, there was no correlation between the integral ratio of GAS to SS and Fas, caspase-3 and caspase-8 ($P > 0.05$).

DISCUSSION

LIC arises mainly from mutations in somatic cells. However, conversion of normal to cancer cells is not the result of a single mutation. It is achieved through a multi-step process that is closely associated with the accumulation of multiple gene changes including both oncogenes and tumor suppressor genes[12-16]. Uncontrolled cell proliferation and apoptosis are the main hallmarks of LIC. Apoptosis is a unique physiological mechanism that it can not only eliminate discrete cells in normal development, host defense and maintain the body in well stable condition, also play an important role in regulating and controlling tumor occurrence, development and treatment[17,18]. Impaired apoptosis has been implicated in the development of many human diseases, including cancer. It has been proved that occurrence of cancers is due to the loss of control of normal apoptosis and the disturbance of balance between cell proliferation and apoptosis[19-21]. Two main signaling pathways initiate the apoptotic program in mammalian cells[22]. The cell-extrinsic pathway, namely, death receptor signaling pathway triggers apoptosis in response to activation by their respective ligand of the tumor necrosis factor (TNF) family of death receptors, including TNF-RI for TNFa, Fas or CD95 for FasL, and death receptor 4 (DR4) or 5 (DR5) for TNF-related apoptosis-inducing ligand (TRAIL). On the other hand, the cell-intrinsic pathway, namely, mitochondria signaling pathway triggers apoptosis in response to DNA damage, loss of survival factors, or other types of cell distress. Both pathways involve the activation of cysteine proteases called caspased, which are constitutively expressed in the cytosol as proenzymes, and are activated to mature proteases by cleavage, which triggers the activation of the caspase-8 and caspase-9, followed by the activation of caspase-3. Caspase-3 is one of the key executioners in the apoptosis pathway. Caspase-3 activation is thought to be a major step in the apoptotic signal transduction cascade that commits cells to suicide. Caspase-3 cleavage during apoptosis leads to the proteolysis of several cytosolic and nuclear proteins and precedes DNA fragmentation. However, some recent studies have shown that activation of Fas/FasL signaling pathway is regulated by many kinds of cell factors, including GAS and SS[23-25].

Many studies have pointed out that some tissue growths are regulated by hormones, and these tissues turned into tumors are still controlled by hormones[26-28]. Gastrointestinal hormones such as GAS and SS regulate the secretion, motility, absorption, blood flow and cell nutrition of the digestive tract. Abnormality of their secretion often affected the normal functions of digestive tract, even produced clinical symptoms or syndromes[29]. Xie *et al*[30] found that the disordered gastrointestinal hormones play a crucial role in the pediatric chronic gastritis and duodenal ulcer. There seems to be an increasing tendency in the expressions of GAS and SS in children with chronic gastritis and duodenal ulcer. In recent years, some studies have demonstrated that there is a high correlation between the aberrant expressions of GAS, SS and the occurrence and development of LIC[31-33]. D'Onghia *et al*[34] found that GAS serum levels were slightly higher in patients with colorectal cancer than in healthy controls. GAS levels were higher in patients carrying left colorectal cancer than the others, and higher in early stage than in late stage. Cao *et al*[35] found that GAS 17 can increase colorectal cancer cells' invasion, the mechanism of which is probably that GAS 17 makes FAK-Tyr397 to be phosphorylated and localized to lamellipodia, causing the formation of FAK-Src-p130 (Cas)-Dock180 signaling complex when it is bound to its receptor CCK-2 and activation of Rac. In this study, we found that there was a significant positive correlation between mRNA and protein expression of GAS and SS. The positive expression rate of GAS and SS mRNA and protein varied greatly in different histological type, differentiation degree, and Dukes' stage. The higher mRNA and protein expression of GAS was, the poorer of tissue differentiation degree and clinical stages.

For example, the expression GAS in poorly differentiated LIC, in particular, in mucinous adenocarcinomas and signet-ring cell carcinoma was prominently higher than the others, with poor prognosis. However, the action of SS was opposite. The results indicate that the expressions of GAS and SS were closely related to the biological behavior of LIC. It is obvious that the GAS and SS will be valuable targets of the biological behavior of LIC.

Tumor escape mechanisms in LIC result from dysregulation of the cell apoptosis signaling pathways, which are mediated by death ligands. Recently, great progress has been made in understanding the regulated signaling pathway mechanisms of GAS and SS. Some studies showed that the abnormal expressions of GAS and SS were closely related to cell apoptosis of LIC. GAS could promote cell proliferation and inhibit cell apoptosis. However, the action of SS was opposite in LIC[36,37]. Our studies showed that the expression of GAS protein in LIC was higher and the AI was lower, and the higher expression of SS protein, the higher AI. Despite abundant evidence that GAS may play an integral role in promoting tumor growth in the stomach and malignancies in the GI tract, the precise mechanisms about gastrin-restrained, somatostatin-induced apoptosis are still largely unknown. To elucidate the mechanisms by which GAS and SS might influence apoptotic signaling pathway, we analyzed them effects on the expression of Fas, FasL, caspase-3 and caspase-8 protein in human LIC tissue.

GAS is a gastrointestinal (GI) peptide that possesses potent trophic effects on most of the normal and neoplastic mucosa of the GI tract. GAS is mainly secreted from GAS secreting cells (G cells) in the antrum mucosa or upper small intestine and large intestine. Medulla oblongata and dorsal nuclei of vagus nerves in central nervous system also secrete GAS[38,39]. Recent reports indicate that chronic hypergastrinaemia increases the risk of colorectal cancer and cancer growth, and that interruption of the effects of GAS could be a potential target in the treatment of colorectal cancer[40]. Recent data have shown that GAS is not only able to induce cell proliferation, but also inhibit LIC cell apoptosis. However, the underlying mechanisms that GAS inhibits cell apoptosis, is still worthy of further elucidation. Wu *et al*[41] recent studies found that down-regulation of GAS gene expression results in the activation of caspase 9 and 3 in gastrin-dependent human colon cancer cells inducing cell apoptosis. Cui *et al*[42] found that GAS can induce apoptosis in gastric epithelial cells via GAS/CCK-2 receptor and synergized with FasL stimulation and contribute to the development of gastric carcinogenesis. In this study, we found that the level of GAS protein expression was higher and the positive expression rate of FasL was higher in LIC tissues, and expression of GAS protein was not related to expression of Fas, caspase-3 and caspase-8. The results indicate that the mechanisms of GAS inhibiting LIC cell apoptosis might be *via* up-regulation of expression of FasL inducing apoptosis with Fas-expressing T cells, making LIC cells escape from immune surveillance and immunocyte attack.

SS is a multifunctional hormone, which is secreted from SS secreting cells (D cells). D cells are distributed mainly in human central and peripheral nervous system, and in the gastrointestinal tract, including the large intestine. SS inhibits multiple functions, including exocrine and endocrine secretions, inflammation, and angiogenesis, as well as cell proliferation and tumorigenesis, as shown in normal or tumoral cell models[43-46]. SS acts as an inhibitory peptide of various secretory and proliferative responses. The diverse biological effects of SS are mediated through a family of five SS receptors (sst1-sst5) that belong to the family of G-protein-coupled receptors and that regulate diverse signal transduction pathways including adenylate cyclase, phospholipase C-β, phospholipase A2, guanylate cyclase, ionic conductance channels, and tyrosine phosphatase[47,48]. The mechanisms of the inhibition are the combined interaction of SS and its analogs with SST1-5R in tumor tissues, either directly inhibiting division and proliferation of tumor cells or inhibiting the activities of growth factors such as vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF), *etc*[49-51], thus counteracting tumorigenesis and tumor cell proliferation. The ability of SS and its stable analogues to promote tumor cell apoptosis has been demonstrated in various cell types including mammary, prostatic, gastric, pancreatic, colorectal, and small cell lung cancer cells. However, the underlying mechanisms of SS induced cell apoptosis are still poorly understood. Guillermet *et al*[52] showed that sst2-dependent activation and cell sensitization to death ligand-induced apoptosis involved activation of the executioner caspases are key factors in both death ligand-or mitochondria-mediated apoptosis. Sst2 affected pathways by up-regulating expression of TRAIL and TNFalpha receptors, DR4 and TNFRI, respectively, and sensitizing the cells to death ligand-induced initiator capase-8 activation; by down-regulating expression of the antiapoptotic mitochondrial Bcl-2 protein; and by enhancing TNFalpha-mediated activation of NF-kappaB, resulting in JNK inhibition and subsequent executioner caspase activation and cell death. Kang *et al*[53] found that SS was able to induce peritoneal macrophages apoptosis by a Bax- and NO-independent p53 accumulation, and through Fas and caspase-8 activation pathways. Recent reports have demonstrated that SS analogs octreotide was able to induce human somatotroph tumor cell apoptosis by activating SST2 and also induce an increase in caspase-3 levels[54,55]. In this study, we found that the higher the integral of SS was, the higher the positive expression rate of Fas, caspase-3, caspase-8, and expression of SS protein was foreign to expression of FasL. Our results indicated that SS was able to induce over-expression of Fas, caspase-3 and caspase-8 protein, and the mechanisms of SS promoting LIC cell apoptosis can be *via* up-regulation of expression of Fas or through increasing caspase-3 levels and sensitivity of death receptor signaling pathway.

In this study, we found that the ratio of GAS to SS had an effect on biological characteristics such as malignant type, tissue differentiation and clinical stages of LIC. The increased ratio of GAS to SS is an event of significance in LIC occurrence and development[56,57]. Our results indicated that there was a positive correlation between the ratio of GAS to SS and the semi-quantitative integral of FasL, and negative correlation between the integral ratio of GAS to SS and the AI. Furthermore, the expression of GAS and SS proteins was directly related to the expression of Fas, FasL, caspase-3, 8 in LIC.

In conclusion, the regulation and control of GAS and SS in LIC cell apoptosis may be directly related to the aberrant expression of Fas/FasL. The GAS and SS will be valuable targets of the biological behavior of LIC.

COMMENTS

Background

Although great progress in understanding the molecular aspects of large intestinal cancer (LIC) has been made and several therapeutic agents have been developed, it is still difficult to cure the tumor. The general survival rate of LIC patients does not exceed 40%. Thereby it is highly important to know that what kind of cell factor can influence cell apoptosis, which will enrich the etiology theory of tumor. Despite abundant evidence that gastrin may play an integral role in promoting tumor growth in the large intestine, the precise mechanisms about gastrin-restrained and somatostatin-induced apoptosis are still largely unknown.

Research frontiers

Recent some studies have shown that activation of Fas/FasL signaling pathway is regulated by many kinds of cell factors, including gastrin (GAS) and somatostatin (SS).

Innovations and breakthroughs

Some studies found that GAS was able to inhibit apoptosis in LIC cell, but the role of SS was opposite. However, the detailed molecule mechanism by which GAS and SS mediate cell apoptosis of LIC is not fully known. In this study, the authors analyzed their effects on the expression of Fas, FasL, caspase-3 and caspase-8 protein. To further confirm whether GAS and SS could mediate LIC cell apoptosis mainly via affecting the expression of Fas/FasL, will help us find a new way to treat malignant tumors.

Applications

The GAS and SS will be valuable targets of the biological behavior of LIC.

Terminology

GAS: It is mainly secreted from GAS secreting cells (G cells) in antrum mucosa or upper small intestine and large intestine. Medulla oblongata and dorsal nuclei of vagus nerves in central nervous system also secrete GAS. SS: It is secreted from SS secreting cells (D cells). D cells are distributed mainly in human central and peripheral nervous system, and in the gastrointestinal tract. Fas/FasL: It belongs to the ligand of the tumor necrosis factor (TNF) family of death receptors. Caspase: Mammalian cysteine aspartate proteases (caspase) can be divided into initiator (e.g. caspases 2, 8, 9, 10) and effector (caspases 3, 4, 5, 6, 7, 11, 12, and 13) enzymes.

Peer review

This paper demonstrates that GAS and SS play important roles in the regulation and control of cell apoptosis in LIC, and the mechanism may be directly related to the aberrant expression of Fas/FasL, which will enrich the etiology theory of LIC. In addition, it also provides a way to evaluate targets of the biological behavior of LIC.

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