Correlation between expression of gastrin, somatostatin and cell apoptosis regulation gene bcl-2/bax in large intestine carcinoma

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AIM: To explore the correlation between expression of somatostatin (SS), gastrin (GAS) and cell apoptosis regulation gene bcl-2/bax in large intestine carcinoma.

METHODS: Sixty-two large intestine cancer tissue samples were randomly and retrospectively selected from patients with large intestine carcinoma. Immunohistochemical staining for bcl-2, bax, GAS, SS was performed according to the standard streptavidin-biotin-peroxidase (S-P) method. According to the semi-quantitative integral evaluation, SS and GAS were divided into three groups as follows. Scores 1-3 were defined as the low expression group, 4-8 as the intermediate expression group, 9-16 as the high expression group. Gas and bcl-2 protein expressions in different GAS and SS expression groups of large intestine carcinoma were assessed.

RESULTS: The positive expression rate of bax had a prominent difference between SS and GAS high, intermediate and low expression groups ($\chi^2$SS = 9.246; $\chi^2$GAS = 6.981). The positive expression rate of bax in GAS high (80.0%, 8/10) and intermediate (76.5%, 13/17) expression groups was higher than that in low expression group (40.0%, 14/35) ($\chi^2$GAS low vs high = 6.097). The positive expression rate of bax in GAS high expression group (27.3%, 3/8) was lower than that in low expression group (69.4%, 25/36) ($\chi^2$GAS low vs high = 7.594). However, bax expression in GAS intermediate expression group (46.7%, 7/15) was lower than that in low expression group, but not statistically significant. The positive expression rate of bcl-2 had a prominent difference between SS and GAS high, intermediate and low expression groups ($\chi^2$SS = 7.178; $\chi^2$GAS = 13.831). The positive expression rate of bcl-2 in GAS high (90.9%, 10/11) and intermediate (86.7%, 13/15) expression groups was higher than that in low expression group (44.4%, 16/36) ($\chi^2$GAS low vs high = 7.695). However, the positive expression rate of bcl-2 in SS high (40.0%, 4/10) and intermediate (47.1%, 8/9) expression groups was lower than that in low expression group (77.1%, 27/35) ($\chi^2$SS low vs high = 4.706).

CONCLUSION: The regulation and control of gastrin, somatostatin in cell apoptosis of large intestine carcinoma may be directly related to the abnormal expression of bcl-2, bax.

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Keywords: Large intestine carcinoma; Gastrin; Somatostatin; bcl-2 gene; Bax gene; Apoptosis

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Main reagents
The polyclonal rabbit antibodies against human SS and GAS, monoclonal mouse antibodies against human bcl-2 and bax, and immunohistochemical staining kits were all purchased from Beijing Zhongshan Biological Technology Co, Ltd.

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 bcl-2, bax, GAS, SS was performed according to the standard streptavidin-biotin-peroxidase (S-P) method. The detailed manipulation was conducted according to the introductions for users. A previously known positive pancreatic tissue, stomach antrum mucous membrane, amygdala tissue, Hodgkin’s disease tissue were used as positive controls for GAS, SS, bcl-2, bax, respectively. PBS 0.01M wao uged as a negative control to replace the primary antibody.

Evaluation of scores
The standard positive SS and GAS expressions were stained brown-yellow mainly in cell plasma, partly in cell membranes. Both the extent and intensity of immunopositivity of SS and GAS expressions were scored according to Wu et al[8]. The intensity of positivity was scored as follows: 1, no staining; 2, light-yellow; 3, brown-yellow; 4, brown-black. The extent of positivity was scored as follows (one hundred cells were counted by two independent observers, who did not know the clinicopathological features of these large intestine cancers.): 1, ≤5%; 2, >5-10%; 3, >10-20%; 4, >20% of the tumor cells in the respective lesions. The final score was determined by multiplying the intensity with extent of positivity scores, yielding a range from 1 to 16. According to the semi-quantitative integral evaluation, SS and GAS were divided into three groups as follows. Scores 1-3 were defined as the low expression group, 4-8 as the intermediate expression group, 9-16 as the high expression group.

Statistical analysis
Statistical evaluation was performed using chi-square test to differentiate the rates of different groups and using Spearman test to analyze the correlation between the ratio of GAS to SS and the integral of bcl-2 and bax. P<0.05 was considered statistically significant. SPSS 10.0 software for Windows was employed to analyze all data.

RESULTS

Bax expression in GAS, SS high, intermediate, and low expression groups of large intestine carcinoma
The positive expression rate of bax had a prominent difference in SS and GAS high, intermediate and low expression groups of large intestine cancer (P<0.05, χ^2=9.246; P<0.05, χ^2=6.981). The positive expression rate of bax in SS high (80.0%, 8/10) and intermediate (76.5%, 13/17) expression groups was higher than that in low expression group (40.0%, 14/35) (P<0.05, χ^2=5.242; P<0.05, χ^2=6.097). The positive expression rate of bax in GAS intermediate expression group (46.7%, 7/15) was lower than that in low expression group, but without statistical significance (Table 1, Figure 1: A-C).

Figure 1
Strong expressions of GAS, SS, bcl-2 and bax in large intestine carcinoma tissue. A: Strong GAS expression in large intestine carcinoma tissue. S-P×400; B: Strong SS expression in large intestine carcinoma tissue. S-P×400; C: Strong bax expression in SS high expression group of large intestine carcinoma tissue. S-P×200; D: Strong bcl-2 expression in GAS high expression group of large intestine carcinoma tissue. S-P×200.
Table 1 Bax expression in SS and GAS high, intermediate, and low expression groups of large intestine carcinoma

| Groups   | n  | Positive | Negative | Positive rate (%) |
|----------|----|----------|----------|-------------------|
| SS       |    |          |          |                   |
| High     | 10 | 8        | 2        | 80.0 (8/10)*      |
| Intermediate | 17 | 13       | 4        | 76.5 (13/17)*     |
| Low      | 35 | 14       | 21       | 40.0 (14/35)      |
| GAS      |    |          |          |                   |
| High     | 11 | 3        | 8        | 27.3 (3/11)*      |
| Intermediate | 15 | 7        | 8        | 46.7 (7/15)       |
| Low      | 36 | 25       | 11       | 69.4 (25/36)      |

*P<0.05 vs the SS low expression group; †P<0.05 vs GAS low expression group.

**Bcl-2 expression in GAS, SS high, intermediate, and low expression groups of large intestine carcinoma**

The positive expression rate of bcl-2 had a prominent difference in SS and GAS high, intermediate and low expression groups of large intestine cancer (P<0.05, χ²ss = 7.178; P<0.05, χ²GAS = 13.831). The positive expression rate of bcl-2 in GAS high (90.9%, 10/11) and intermediate (86.7%, 13/15) expression groups was higher than that in low expression group (44.4%, 16/36) (P<0.05, χ² high vs low = 5.600; P<0.05, χ² middle vs low = 7.695). However, the positive expression rate of bcl-2 in SS high (40.0%, 4/10) and intermediate (47.1%, 8/9) expression groups was lower than that in low expression group (77.1%, 27/35) (P<0.05, χ² high vs low = 4.710; P<0.05, χ² middle vs low = 4.706) (Table 2, Figure 1: D).

Table 2 Bcl-2 expression in SS and GAS high, intermediate, and low expression groups of large intestine carcinoma

| Groups   | n  | Positive | Negative | Positive rate (%) |
|----------|----|----------|----------|-------------------|
| SS       |    |          |          |                   |
| High     | 10 | 4        | 6        | 40.0 (4/10)*      |
| Intermediate | 17 | 8        | 9        | 47.1 (8/17)*     |
| Low      | 35 | 27       | 8        | 77.1 (27/35)     |
| GAS      |    |          |          |                   |
| High     | 11 | 10       | 1        | 90.9 (10/11)*    |
| Intermediate | 15 | 13       | 2        | 86.7 (13/15)     |
| Low      | 36 | 16       | 20       | 44.4 (16/36)     |

*P<0.05 vs the SS low expression group; †P<0.05 vs the GAS low expression group.

**Correlation between the integral ratio of GAS to SS and the integral of bcl-2/bax**

There was a significant positive correlation between the integral ratio of GAS to SS and the integral of bcl-2 (P<0.01, r = 0.340). However, there was a negative correlation between the integral ratio of GAS to SS and the integral of bax (P<0.05, r = -0.299).

**DISCUSSION**

Previous studies have shown that tissue growth is regulated by hormones, and their tumor growth and development are still controlled by hormones[10]. Gastrointestinal hormones such as gastrin and somatostatin regulate the secretion, motility, absorption, blood flow and cell nutrition of the digestive tract. Abnormality of their secretion often affects the normal functions of digestive tract, even causes clinical symptoms or syndromes[11,12]. Some studies have demonstrated that there is a high correlation between the abnormal expressions of GAS, SS and the occurrence and development of large intestine cancer[13-15]. Recent studies have shown that the abnormal expressions of GAS and SS are closely related to cell apoptosis of large intestine cancer. Gastrin could promote cell proliferation and inhibit cell apoptosis. However, the action of somatostatin is opposite in large intestine carcinoma[16,17].

Apoptosis can not only maintain the body in well stable condition, but also plays an important role in regulating and controlling tumor occurrence, development and treatment[18]. It has been proved that occurrence of cancer is due to the loss of control of normal apoptosis and the disturbance of balance between cell proliferation and apoptosis[19,20]. Apoptosis related genes such as bcl-2 family are divided into two categories: pro-apoptosis genes and anti-apoptosis genes. Bcl-2 is an important apoptosis repressor, while Bax is one of the most important apoptosis promoters. The protein it encodes could combine with Bcl-2 to form compounds, which resist the action of apoptosis repression. But it has a positive regulatory action[21-26]. Recent data indicate that the regulation and control of cell apoptosis by bax and bcl-2 genes are not only based on the level of the two regulatory proteins but also based on their ratio. When the ratio is high, cells undergo apoptosis, otherwise, they proliferate[22,25,26].

Gastrin is mainly secreted from gastric secreting cells (G cells) in antrum mucosa or upper small intestine, large intestine. Medulla oblongata and dorsal nuclei of vagus nerves in central nervous system also secrete gastrin[27]. Some studies indicate that external gastrin is able to inhibit apoptosis of MKN45 cells by inducing over-expression of anti-apoptosis gene bcl-2, and prolumide could block these effects of gastrin[28]. Zhang et al[29] found that gastrin was able to increase the threshold of apoptosis by upregulating bcl-2 gene expression in human cholangiocarcinoma cells, but it had no effect on the expression of bax gene. However, Hartwich et al[30] found that gastrin was able to restrain the apoptosis of tumor cells by inducing over-expression of bcl-2 and inhibiting the bax gene activity. Whether gastrin can regulate and control cell apoptosis mainly by affecting the bax gene expression, needs further studies. In this study, we found that GAS protein expression and the positive expression rate of bcl-2 were higher in large intestine carcinoma, however the expression of bax protein was opposite. These results accord with those of foreign reports and indicate that GAS regulation and control of cell apoptosis and proliferation of large intestine cancer can induce over-expression of bcl-2 protein and down-regulate bax gene activity.

Somatostatin is distributed in human hypothalamus and other sites of the brain, peripheral nerve and gastrointestinal tract. In the digestive system, for example, somatostatin is secreted from somatostatin secreting cells (D cells). D cells are distributed mainly in intestinal nerve plexus, stomach and pancreas. Somatostatin acts as an inhibitory peptide of various secretory and proliferative responses. It has been found that its effects are mediated by a family of G-protein-coupled receptors (sst1-5), etc[35-37], thus counteracting tumorigenesis and tissue proliferation[38]. Recent data have shown that somatostatin is not only able to restrain cell proliferation, but also to induce tumour cell apoptosis. However, the underlying mechanisms have not been elucidated. Sharma et al[39] reported that somatostatin analogs (SSAs) octreotide (OCT) could elicite cytotoxic response in MCF-7 human breast cancer cells, leading to apoptosis which is associated with a rapid, time-dependent induction of wild-type.
p53 and an increase of bax. Kang et al[10] demonstrated that apoptosis by somatostatin might occur due to bax- and NO-independent p53 accumulation, and through Fas and caspase-8 activation pathways in peritoneal macrophages. Yuan et al[11] found that somatostatin analogs (SSa) were able to induce the apoptosis of pancreatic acinar cells. The mechanisms of apoptosis are probably correlated with the expression of apoptosis-regulated gene bax, but have no relationship with the expression of p53. In a word, somatostatin and its analogues could induce cell apoptosis. In this study, we found that the higher the integral of SS was, the higher expression of bax protein, but the expression of bcl-2 protein was lower. Our data indicate that SS can promote cell apoptosis and restrain cell proliferation of large intestine carcinoma. The mechanism is through up-regulation of bax gene expression and inhibition of the activity of anti-apoptosis gene bcl-2.

In the present 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 large intestine cancer. The ratio of GAS to SS was increased, which is of significance in diagnosis [12] and staging of colorectal cancer. In the present study, we found that the ratio of GAS to SS and the semi-quantitative integral of bcl-2 expression, and a negative correlation between GAS/SS and bax. Furthermore, the expression of GAS and SS proteins has a direct relation with the expression of bax and bcl-2.

In conclusion, abnormal expression of GAS and SS can lead to abnormal expression of bcl-2 and bax in large intestine carcinoma.

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