Correlation between the expressions of gastrin, somatostatin and cyclin and cyclin-depend kinase in colorectal cancer

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AIM: To explore the correlation between the expressions of gastrin (GAS), somatostatin (SS) and cyclin, cyclin-depend kinase (CDK) in colorectal cancer, and to detect the specific regulatory sites where gastrointestinal hormone regulates cell proliferation.

METHODS: Seventy-nine resected large intestine carcinomatous specimens were randomly selected. Immunohistochemical staining for GAS, SS, cyclin D1, cyclin E, cyclin A, cyclin B1, CDK2 and CDK4 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 high, middle and low groups. Cyclin D1, cyclin E, cyclin A, cyclin B1, CDK2, CDK4 expressions in the three GAS and SS groups were assessed.

RESULTS: The positive expression rate of cyclin D1 was significantly higher in high (78.6%, 11/14) and middle GAS groups (73.9%, 17/23) than in low GAS group (45.2%, 19/42) (P<0.05, χ²high vs low = 4.691; P<0.05, χ²middle vs low = 4.945). The positive expression rate of cyclin A was significantly higher in high (100%, 14/14) and middle GAS groups (82.6%, 19/23) than in low GAS group (54.8%, 23/42) (P<0.01, χ²high vs low = 9.586; P<0.05, χ²middle vs low = 5.040). The positive expression rate of CDK2 was significantly higher in high (92.9%, 13/14) and middle GAS groups (87.0%, 20/23) than in low GAS group (50.0%, 21/42) (P<0.01, χ²high vs low = 8.086; P<0.01, χ²middle vs low = 8.715). The positive expression rate of CDK4 was significantly higher in high (78.6%, 11/14) and middle GAS groups (78.3%, 18/23) than in low GAS group (42.9%, 18/42) (P<0.05, χ²high vs low = 5.364; P<0.01, χ²middle vs low = 7.539). The positive expression rate of cyclin E was prominently higher in low SS group (53.3%, 24/45) than in high (9.1%, 1/11) and middle (21.7%, 5/23) SS groups (P<0.05, χ²high vs low = 5.325; P<0.05, χ²middle vs low = 6.212). The positive expression rate of CDK2 was significantly higher in low SS group (77.8%, 35/45) than in high SS group (27.3%, 3/11) (P<0.01, χ²high vs low = 8.151). There was a significant positive correlation between the integral ratio of GAS to SS and the semi-quantitative integral of cyclin D1, cyclin E, cyclin A, CDK2, CDK4 (P<0.05, ρ = 0.252; P<0.01, ρ = 0.387; P<0.01, ρ = 0.466; P<0.01, ρ = 0.519; P<0.01, ρ = 0.434).

CONCLUSION: The regulation and control of gastrin, SS in colorectal cancer cell growth may be directly related to the abnormal expressions of cyclins D1, A, E, and CDK2, CDK4. The regulatory site of GAS in the cell cycle of colorectal carcinoma may be at the G1, S and G2 phases. The regulatory site of SS may be at the entrance of S phase.

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Key words: Colorectal cancer; Gastrin; Somatostatin; Cyclin; CDK

INTRODUCTION

Colorectal cancer is one of the most common human 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[1-3]. Although great progress has been made in understanding the molecular aspects of colorectal cancer and several therapeutic agents have been developed, it still poses a serious threat to public health and remains as the major killer among the Chinese. The general survival rate of colorectal cancer patients does not exceed
Studies demonstrate that the occurrence of colorectal cancer is directly related to the abnormal expression of gastrointestinal hormones such as gastrin, somatostatin, etc. At the same time, some studies found that somatostatin is able to induce apoptosis of large intestinal cancer cells and inhibit cell proliferation, but the role of GAS (gastrin) is opposite. However, the detailed molecule mechanism by which gastrin and somatostatin regulate and control the growth of large intestinal carcinoma is not fully known. We have used immunohistochemical staining standard streptavidin-biotin-peroxidase (S-P) method to detect the expressions of GAS, somatostatin (SS), cyclin D1, cyclin E, cyclin A, cyclin B1, CDK2, CDK4 proteins in large intestinal cancer tissue. The aim of our study was to explore the correlation between the expressions of SS, GAS and cyclins, cyclin-dependent kinase (CDKs) and to further confirm whether GAS, SS could regulate and control large intestinal cancer cell growth by affecting the expression of cyclins and CDKs.

MATERIALS AND METHODS

Seventy-nine cancer tissue samples were randomly selected from patients with large intestinal carcinoma hospitalized in the Department of Pathology of the First Affiliated Yijishan Hospital of Wannan Medical College from June 2001 to June 2003. All patients were confirmed to have colorectal carcinoma by clinical pathology. Among them, 37 were cases of rectum cancer, 42 were cases of colorectal carcinoma. Thirty-five were females and 44 were males. The median age was 52.9 ± 14.3 years, with a range of 27-78 years. Ulcerative type was found in 44 patients, protruded type in 33, infiltrating type in 2, papillary adenocarcinoma in 7, glandular adenocarcinoma in 40, mucoid carcinoma in 14, signet-ring cell carcinoma in 11, and undifferentiated carcinoma in 7. The clinical stage was determined according to the Dukes’ stage. Dukes’ stages A and B were found in 38 patients, Dukes’ stages C and D in 41 patients.

The polyclonal rabbit antibodies against human SS and GAS, monoclonal mouse antibodies against human cyclins D1, E, A, B1, and CDK2, CDK4, and immunohistochemical staining kits were all purchased from Beijing Zhongshan Biological Technology Co., Ltd.

Specimens obtained during surgery were routinely fixed in 10% neutral formalin and embedded in paraffin. Serial 4-μm-thick sections were cut. Immunohistochemical staining for cyclins D1, E, A, B1, and CDK2, CDK4, GAS, SS was performed according to the S-P method. The detailed manipulation was conducted according to the introduction for users. Positive pancreatic tissue, stomach antrum mucous membrane, healthy amygdalae tissue, breast cancer tissue, reactive lymph node tissue, healthy skin tissue were used as a positive control for GAS, SS, cyclins A, B1, D1, E, and CDK2, CDK4, respectively. PBS (0.01mol/L) as a negative control replaced the primary antibody.

Positive SS and GAS were stained brown-yellow mainly in cell plasma, partly in cell membranes. When SS and GAS protein expression were scored, both the extent and intensity of immunopositivity were considered. The intensity of staining was scored as follows: 0 as no staining, 1 as weak-yellow, 2 as brown-yellow, and 3 as 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 large intestinal cancers): 1 = positively stained cells <5%, 2 = positively stained cells being 5-10%, 3 = positively stained cells being 10-20%, 4 = positively stained 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 as follows: scores 1-3 were defined as the low group, 4-6 as the middle group, and 7-12 as the high group.

Positive cyclin B1 was stained brown-yellow mainly in the cell plasma. Positive cyclins D1, E, A, and CDK2, CDK4 were stained brown-yellow mainly in karyons. The degree of their staining was estimated by semiquantitative evaluation and categorized by the extent and intensity of staining as follows. The intensity of staining was scored as follows: 0 as negative, 1 as weak-yellow, 2 as brown-yellow, and 3 as brown-black. The extent of positively stained cells was scored as follows: 0=positively stained cells being 0-5%, 1 = positively stained cells being 6-25%, 2 = positively stained cells being 26-50%, 3 = positively stained cells being 51-75%, 4 = positively stained cells>75%. Combined staining score was used to evaluate the staining of cyclins D1, E, A, B1, and CDK2, CDK4. The final score was determined by adding the intensity and extent of staining scores, yielding a range from 0 to 7. Scores1-2 were defined as negative staining (-), 3 as weak staining (+), 4 as moderate staining (++), 5 as strong staining (+++).

Statistical analysis was performed using chi-square test to differentiate the positive rates of different groups and using Spearman’s test to analyze the correlation between the ratio of GAS to SS and the integral of cyclins D1, E, A, B1, and CDK2, CDK4. All data were analyzed with SPSS version 10.0. P < 0.05 was considered statistically significant.

RESULTS

The positive expression rate of cyclin D1 was significantly higher in high (78.6%, 11/14) and middle GAS groups (73.9%, 17/23) than in low GAS group (45.2%, 19/42) (P < 0.05, \( \chi^2 \) high vs low = 4.691; P < 0.05, \( \chi^2 \) middle vs low = 4.945). The positive expression rate of cyclin A was significantly higher in high (100%, 14/14) and middle GAS groups (82.6%, 19/23) than in low GAS group (54.8%, 23/42) (P < 0.01, \( \chi^2 \) high vs low = 9.586; P < 0.05, \( \chi^2 \) middle vs low = 5.040). The positive expression rate of CDK2 was significantly higher in high (92.9%, 13/14) and middle GAS groups (87.0%, 20/23) than in low group (50.0%, 21/42) (P < 0.01, \( \chi^2 \) high vs low = 8.086; P < 0.01, \( \chi^2 \) middle vs low = 8.715). The positive
expression rate of CDK4 was significantly higher in high (78.6%, 11/14) and middle GAS groups (78.3%, 18/23) than in low group (42.9%, 18/42) ($P<0.05$, $\chi^2_{high \ vs. \ low} = 5.364$; $P<0.01$, $\chi^2_{middle \ vs. \ low} = 7.539$). However, the positive expression rate of cyclins E and B1 was significantly higher in high (57.1%, 8/14; 92.9%, 13/14) and middle GAS groups (47.8%, 11/23; 73.9%, 17/23) than in low GAS group (26.2%, 11/42; 66.9%, 28/42), but there was no statistically significant difference among the three groups when compared to three groups to each other ($P>0.05$, $\chi^2 = 5.608$; $P>0.05$, $\chi^2 = 4.417$) (Table 1, Figure 1 A-D).

The positive expression rate of cyclin E was prominently higher in low SS group (53.3%, 24/45) than in high (9.1%, 1/11) and middle (21.7%, 5/23) SS groups ($P<0.05$, $\chi^2_{high \ vs. \ low} = 5.325$; $P<0.05$, $\chi^2_{middle \ vs. \ low} = 6.212$). The positive expression rate of CDK2 was significantly higher in low SS group (77.8%, 35/45) than in high SS
group (27.3%, 3/11) (P<0.01, \( \chi^2_{\text{high} \times \text{low}} = 8.151 \)). However, the positive expression rate of CDK4, cyclin D1 was significantly higher in low SS group (64.4%, 29/45; 62.2%, 28/45) than in high SS group (36.4%, 4/11; 45.5%, 5/11), it was not statistically significant (\( P>0.05, \chi^2 = 2.868; P>0.05, \chi^2 = 1.038 \)). There was no statistically significant difference in the positive expression rate of cyclins A and B1 in high (72.2%, 8/11; 81.8%, 9/11), middle (69.6%, 16/23; 73.9%, 17/23) and low SS groups (71.0%, 32/45; 71.1%, 32/45) when compared to each other (\( P>0.05, \chi^2 = 0.039; P>0.05, \chi^2 = 0.554 \)) (Table 1, Figure 1 E-G).

There was a significant positive correlation between the integral ratio of GAS to SS and the semiquantitative integral of cyclins D1, E, A, and CDK2, CDK4 (\( P<0.05, \chi^2 = 0.252; P<0.01, \chi^2 = 0.387; P<0.01, \chi^2 = 0.466; P<0.01, \chi^2 = 0.519; P<0.01, \chi^2 = 0.434 \)). But there was no correlation between the integral ratio of GAS to SS and cyclin B1 integral (\( P>0.05, r = -0.108 \)).

**DISCUSSION**

Cancer arises mainly from mutations in the somatic cells. However, conversion of normal cells 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\(^{11-13}\). Uncontrolled cell proliferation is one of the main hallmarks of cancer, and tumor cells have acquired damage to genes that are directly involved in regulating the cell cycle. The cell cycling process is carefully regulated. The switch or transition between phases is a hallmark of the cell cycle, with an extremely accurate timing and order of molecular events. However, if something goes wrong, the cells have several systems for interrupting the cell cycle\(^{12,14,15}\).

In order to ensure the cell cycling process, CDK timing-activity is a critical step in the regulation and control mechanism of cell cycle. At least nine different CDKs are known today. However, some of them seem to be involved in cell cycle regulation. CDKs that are required for cell cycle regulation contain an active kinase subunit in complex with a regulatory subunit, or activator, commonly called cyclin\(^{16,17}\). Cyclins are important mediators of CDKs activity, and their level fluctuates throughout the cell cycle, some being more abundant in specific cell phases than others\(^{18}\). These cyclins have been divided into three classes: G1-S cyclin, S cyclin, and M cyclin. Cyclins response to mitogenic signals and unscheduled expression, leading to uncontrolled proliferation, has been implicated in different human cancers\(^{19}\), such as colon cancer\(^{20}\) and breast cancer\(^{21}\). The CDK/cyclin complex is subjected to several kinds of regulation factors, both positive and negative.

Cell cycle progression is positively regulated by multiple cyclins and CDKs and cyclin/CDK complexes are negatively regulated by a number of CDK inhibitors including p27\(^{22,23}\). P27 is a CDK inhibitor and plays an important role in the negative regulation of the cell cycle during G0 and G1 phases\(^{22,13,24,25}\). Proliferating cells pass through several cell cycle checkpoints, mainly the G1 to S and G2 to M transitions. The former checkpoint is considered as the most important one in the replication of DNA and mitosis. The G1-S transition is a highly regulated and important transition in the cell cycle. At this stage, the cell cycle passes a point between G1 and S phases (restriction point) with an irreversible commitment to a new cycle. The underlying molecular mechanisms are the induced expression of CDKs and cyclins required for the cells to progress from early G1 phase into late G1 phase of the cell cycle, reaching the restriction point. This is a critical point in the late G1 phase where the mammalian cells become committed to entering the S phase and to complete the cell cycle, even in the absence of growth factor\(^{23,26,27}\). The main CDKs involved in the progression from mid- to late G1 are CDK4 and CDK6, driven by three G1-specific cyclins, D1, D2 and D3\(^{28}\). Cycle progression from G1 to S phase is usually accompanied with Rb phosphorylation induced by cyclin D1-CDKs and cyclin E-CDK2 complexes in the late G1 phase\(^{29}\). Cyclin-dependent kinase 2 (CDK2) activity is critical for S phase entry. CDK2 activation is apparently cyclin E-dependent. Late G1 phase CDK2/cyclin E activity depends on early G1 phase CDK2/cyclin E function\(^{29,30}\).

Previous studies have shown that some tissue growth is regulated by hormones, and these tissues that have turned into tumors are still controlled by hormones\(^{31}\). 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 the digestive tract, even causes clinical symptoms or syndromes\(^{32,33}\). Some studies demonstrated that there is a high correlation between the abnormal expressions of GAS, SS and the occurrence and development of colorectal cancer\(^{34-36}\). Recently, great progress has been made in understanding the cell cycle mechanisms of GAS and SS. Some studies showed that the abnormal expressions of GAS and SS are closely related to cell apoptosis and proliferation of colorectal cancer, and that the expression of gastrin protein and the proliferation index are higher in colorectal carcinoma\(^{9,37,38}\). Though there is abundant evidence that gastrin plays an important role in promoting tumor growth in the stomach, as well as malignancies in the GI tract, the precise mechanisms governing the gastrin-induced and somatostatin-restrained proliferation are still largely unknown. To elucidate the mechanisms of gastrin and somatostatin in regulating mitogenesis, we have analyzed their effects on the expression of cyclins and CDKs in human colorectal cancer tissue.

Gastrin is a gastrointestinal (GI) peptide that possesses potent trophic effects on most normal and neoplastic mucosa of the GI tract. Gastrin is mainly secreted from gastrin secreting cells (G cells) in the antrum mucosa or upper small intestine, large intestine. Medulla oblongata and dorsal nuclei of vagus nerves in central nervous system also secrete gastrin\(^{39,40}\). Studies indicate that
chronic hypergastrinemia increases the risk of colorectal cancer and cancer growth, and that interruption of the effects of gastrin may be a potential target in the treatment of colorectal cancer. Shen et al. showed that gastrin is able to promote DNA and protein synthesis in colorectal cancer tissue. However, gastrin-released peptide receptor antagonist proglumide could block these effects of gastrin, and restrain colorectal cancer cells from G_{0}/G_{1} phase into S and G_{2}/M phase transitions. It was recently reported that gastrin (G-17) is able to induce a significant increase in G_{1}-specific marker cyclin D1 transcripts, protein, and promoter activity via the activation of beta-catenin and CRE-binding protein pathways in gastric adenocarcinoma cells, which promote transition of tumor cells from G_{1} phase into S phase, and lead to uncontrolled proliferation of tumor cells. Lefranc et al. studies found that gastrin is able to significantly modify the growth of a number of experimental gliomas. This effect seems to occur via a cytostatic effect, that is, an accumulation of tumor astrocytes occurs in the G_{1} cell cycle phase. The cytostatic effect relates to a gastrin-induced decrease in the level of cyclin D3-CDK4 complex. In this study, we have found that the level of gastrin protein expression was higher and the positive expression rate of cyclins D1, A, and CDK2, CDK4 was higher in large intestinal carcinoma tissue, indicating that mechanism of gastrin in promoting colorectal cancer cell proliferation is via inducing the overexpression of cyclins D1, A, and CDK2, CDK4, thus leading to the rise of the level of cyclin D1-CDK4 and cyclin A-CDK2 complexes, which influence cell cycle progress and promote cell proliferation.

Somatostatin is a widely distributed inhibitory hormone that plays an important role in several biological processes including neurotransmission, inhibition of exocrine and endocrine secretions, and cell proliferation. Somatostatin acts as an inhibitory peptide of various secretory and proliferative responses. Somatostatin is secreted from somatostatin secreting cells (D cells). D cells are distributed mainly in intestinal nerve plexus, stomach and pancreas. The diverse biological effects of somatostatin are mediated by a family of five somatostatin receptors (sst1-sst5) that belong to the family of G-protein-coupled receptors and regulate diverse signal transduction pathways including adenylate cyclase, phospholipase C-β phospholipase A_{2}, guanylate cyclase, ionic conductance channels, and tyrosine phosphatase. The mechanisms underlying the inhibition are the combined interaction of somatostatin and its analogs to SST1-5R in tumor tissues, either directly inhibiting division and proliferation of tumor cells or the activities of growth factors such as vascular endothelial growth factor, insulin-like growth factor, etc., thus counteracting tumorigenesis and tumor cell proliferation. The ability of somatostatin and its stable analogs to inhibit normal and tumor cell growth has been demonstrated in various cell types including mammary, prostatic, gastric, pancreatic, colorectal, and small cell lung cancer cells. However, the mechanisms of somatostatin underlying cell growth arrest are still poorly understood.

Pages et al. showed that activation of sst2 promotes cell growth arrest through the effect of somatostatin to maintain high levels of p27^{kip1} and inactivates cyclin E-CDK2 complexes, leading to hypophosphorylation of pRb, restraining transition of tumor cells from G_{1} phase into S phase. Charland et al. reported that somatostatin is able to inhibit cyclin E expression in pancreatic cells and cyclin E-associated CDK2 activity, as well as pRb phosphorylation, and to restrain transition of cells from G_{1} phase into S phase, thus inhibiting cell proliferation. Zuo et al. demonstrated that somatostatin analog, octreotide, inhibits the proliferation of cholangiocarcinoma cells through G_{0}/G_{1} cell cycle arrest rather than through the process of apoptosis. These effects are partially mediated by enhancing the expression of p27^{kip1}, and decreasing the level of cyclin E-CDK2 complex. In this study, the higher the integral of SS, the lower the positive expression rate of cyclin E and CDK2. Our data indicate that the mechanism of somatostatin in inhibiting colorectal cancer cell proliferation is via restraining the expression of cyclin E and CDK2, and decreasing the level of cyclin E-CDK2 complex, which inhibits transition of cells from G_{1} phase into S phase and induces cell cycle arrest, thus restraining cell proliferation.

In the present study, we have found that the ratio of GAS to SS had an effect on the biological characteristics of large intestinal cancer. The increased ratio of GAS to SS is an event of significance in large intestinal cancer occurrence and development. Our results indicate that there is a positive correlation between the ratio of GAS to SS and the semi-quantitative integral expression of cyclins D1, A, E, and CDK2, CDK4. Furthermore, the expression of GAS and SS has a direct relation with the expression of cyclins D1, A, E, and CDK2, CDK4 in colorectal cancer.

In conclusion, the regulation and control of gastrin, somatostatin in colorectal cancer cell growth may be directly related to the abnormal expressions of cyclins D1, A, E, and CDK2, CDK4. The regulatory site of GAS in the cell cycle of colorectal carcinoma may be at the G_{1}, S and G_{2}/M phases. The regulatory site of SS may be at the entrance of S phase.

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