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• *RAPID COMMUNICATION* •

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

Pei Wu, Jia-Ding Mao, Jing-Yi Yan, Jing Rui, You-Cai Zhao, Xian-Hai Li, Guo-Qiang Xu

Pei Wu, Jia-Ding Mao, Jing-Yi Yan, Jing Rui, You-Cai Zhao, Xian-Hai Li, Guo-Qiang Xu, Department of General Surgery, The First Affiliated Yijishan Hospital of Wannan Medical College, Wuhu 241001, Anhui Province, China

You-Cai Zhao, Guo-Qiang Xu, Department of Pathology, The First Affiliated Yijishan Hospital of Wannan Medical College, Wuhu 241001, Anhui Province, China

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Co-first-authors: Pei Wu and Jia-Ding Mao
Co-correspondent: Pei Wu
Correspondence to: Professor Pei Wu, Department of General Surgery, The First Affiliated Yijishan Hospital of Wannan Medical College, Wuhu 241001, Anhui Province, China. wp5708@sina.com
Telephone: +86-0553-5739343

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Abstract

AIM: To explore the correlation between the expressions of gastrin (GAS), somatostatin (SS) and cyclin, cyclin-dependent 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) ($P<0.05$, $\chi^2_{\text{high } vs \text{ low}} = 4.691$; $P<0.05$, $\chi^2_{\text{middle } vs \text{ low}} = 4.945$). 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) ($P<0.01$, $\chi^2_{\text{high } vs \text{ low}} = 9.586$; $P<0.05$, $\chi^2_{\text{middle } vs \text{ low}} = 5.040$). 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) ($P<0.01$, $\chi^2_{\text{high } vs \text{ low}} = 8.086$; $P<0.01$, $\chi^2_{\text{middle } vs \text{ low}} = 8.715$). 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) ($P<0.05$, $\chi^2_{\text{high } vs \text{ low}} = 5.364$; $P<0.01$, $\chi^2_{\text{middle } vs \text{ low}} = 7.539$). 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 ($P<0.05$, $\chi^2_{\text{high } vs \text{ low}} = 5.325$; $P<0.05$, $\chi^2_{\text{middle } vs \text{ low}} = 6.212$). 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) ($P<0.01$, $\chi^2_{\text{high } vs \text{ low}} = 8.151$). 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 ($P<0.05$, ${}^{D1}r_s = 0.252$; $P<0.01$, ${}^{E}r_s = 0.387$; $P<0.01$, ${}^{A}r_s = 0.466$; $P<0.01$, ${}^{K2}r_s = 0.519$; $P<0.01$, ${}^{K4}r_s = 0.434$).

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 G_1, S and G_2 phases. The regulatory site of SS may be at the entrance of S phase.

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

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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

40%[4,5]. Studies demonstrate that the occurrence of colorectal cancer is directly related to the abnormal expression of gastrointestinal hormones such as gastrin, somatostatin, etc[6]. 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[7-9]. 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[10]. 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) ($P<0.05$, $\chi^2_{\text{high } vs \text{ low}} = 4.691$; $P<0.05$, $\chi^2_{\text{middle } vs \text{ low}} = 4.945$). 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) ($P<0.01$, $\chi^2_{\text{high } vs \text{ low}} = 9.586$; $P<0.05$, $\chi^2_{\text{middle } vs \text{ low}} = 5.040$). 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) ($P<0.01$, $\chi^2_{\text{high } vs \text{ low}} = 8.086$; $P<0.01$, $\chi^2_{\text{middle } vs \text{ low}} = 8.715$). The positive

Table 1 CDK2, CDK4, cyclins D1, E, A, and B1 expressions in high, middle, and low SS and GAS groups of colorectal carcinoma

Groups	*n*	Cyclin D1 -	Cyclin D1 + (%)	Cyclin E -	Cyclin E + (%)	Cyclin A -	Cyclin A + (%)	CyclinB1 -	CyclinB1 +(%)	CDK2 -	CDK2 + (%)	CDK4 -	CDK4 + (%)
GAS													
High	14	3	11 (78.6)[a]	6	8	0	14 (100)[b]	1	13(92.9)	1	13 (92.9)[b]	3	11 (78.6)[a]
Middle	23	6	17 (73.9)[a]	12	11	4	19 (82.6)[a]	6	17	3	20 (87.0)[b]	5	18 (78.3)[b]
Low	42	23	19	31	11	19	23	14	28	21	21	24	18
SS													
High	11	6	5	10	1 (9.1)[c]	3	8	2	9	8	3 (27.3)[d]	7	4
Middle	23	9	14	18	5 (21.7)[c]	7	16	6	17	7	16	9	14
Low	45	17	28	21	24	13	32	13	32	10	35	16	29

[a]P<0.05, [b]P<0.01 *vs* low GAS group; [c]P<0.05, [d]P<0.01 *vs* low SS group.

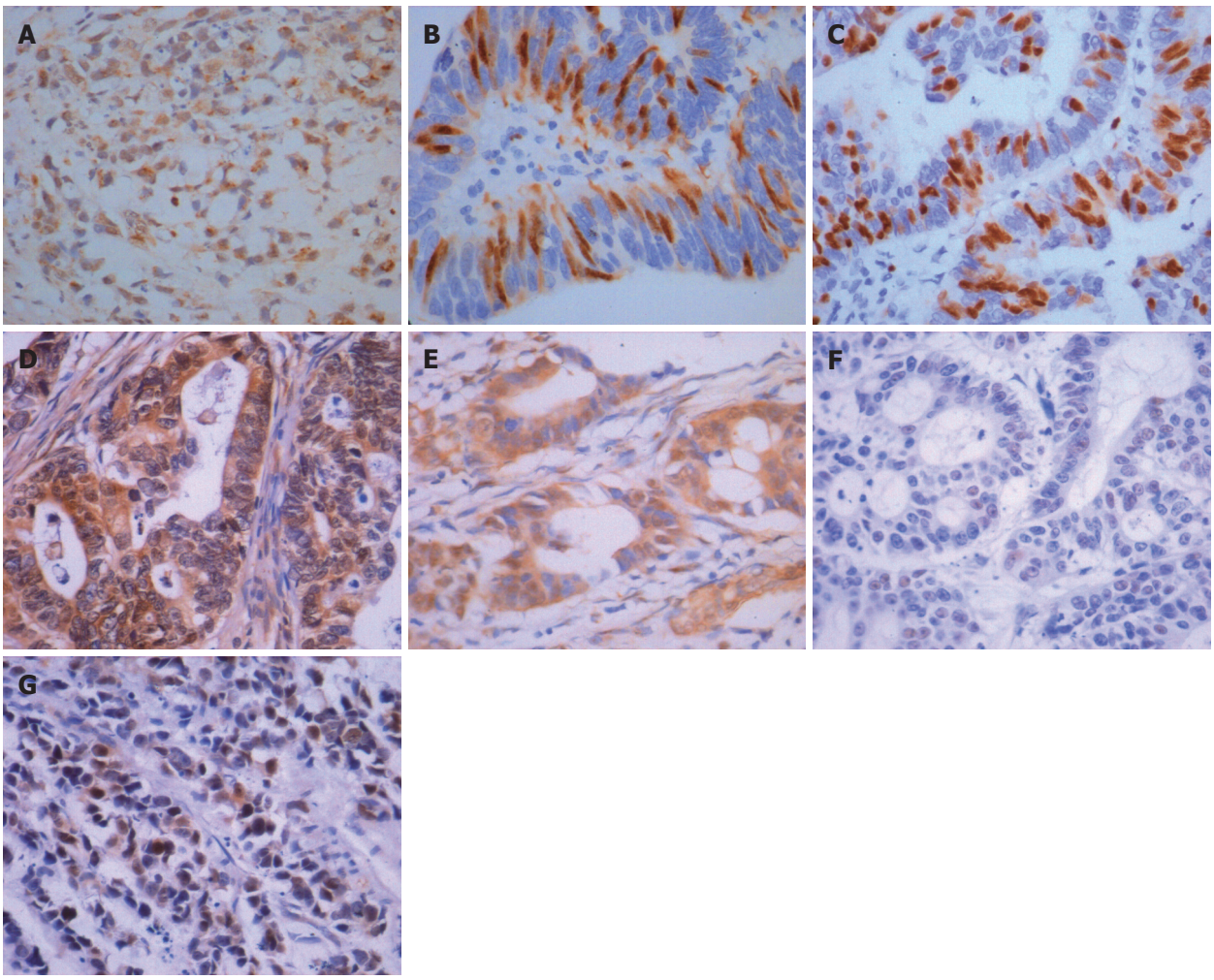

Figure 1 Strong expressions of GAS (**A**), cyclin D1 (**B**), cyclin A (**C**), CDK4 (**D**), SS (**E**), cyclin E (**F**) and CDK2 (**G**) in colorectal carcinoma tissue, S-P×400.

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, $\chi^2_{\text{high vs. low}}$ = 5.364; P<0.01, $\chi^2_{\text{middle vs. low}}$ = 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, χ^2 = 5.608; P>0.05, χ^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, $\chi^2_{\text{high vs low}}$ = 5.325; P<0.05, $\chi^2_{\text{middle vs low}}$ = 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) ($P<0.01$, $\chi^2_{\text{high vs low}} = 8.151$). 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 ($P<0.05$, ${}^{D1}r_s = 0.252$; $P<0.01$, ${}^{E}r_s = 0.387$; $P<0.01$, ${}^{A}r_s = 0.466$; $P<0.01$, ${}^{K2}r_s = 0.519$; $P<0.01$, ${}^{K4}r_s = 0.434$). 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, only 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: G_1-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 G_0 and G_1 phases[12,13,24,25]. Proliferating cells pass through several cell cycle checkpoints, mainly the G_1 to S and G_2 to M transitions. The former checkpoint is considered as the most important one in the replication of DNA and mitosis. The G_1-S transition is a highly regulated and important transition in the cell cycle. At this stage, the cell cycle passes a point between G_1 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 G_1 phase into late G_1 phase of the cell cycle, reaching the restriction point. This is a critical point in the late G_1 phase where the mammalian cells become committed to entering the S phase and to complete the cell cycle, even in the absence of growth factors[23,26,27]. The main CDKs involved in the progression from mid- to late G_1 are CDK4 and CDK6, driven by three G_1-specific cyclins, D1, D2 and D3[15]. Cycle progression from G_1 to S phase is usually accompanied with Rb phosphorylation induced by cyclin D_1-CDK_4 and cyclin E-CDK_2 complexes in the late G_1 phase[28]. Cyclin-dependent kinase 2 (CDK2) activity is critical for S phase entry. CDK2 activation is apparently cyclin E-dependent. Late G_1 phase CDK2/cyclin E activity depends on early G_1 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 cancer tissue, while the action of somatostatin is opposite 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 mucosae 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[41]. Shen *et al*[42] 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[43]. Lefranc *et al*[44] 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[45-48]. The mechanisms underlying the inhibition are the combined interaction of somtostatin 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[49-51], thus counteracting tumorigenesis and tumor cell proliferation[52]. 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*[53] showed that activation of sst2 promotes cell growth arrest through the ability 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*[54] 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. Zhao *et al*[55] 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 p27kipl, 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[56]. The increased ratio of GAS to SS is an event of significance in large intestinal cancer occurrence and development[31]. 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 phases. The regulatory site of SS may be at the entrance of S phase.

REFERENCES

1 **Zhang ZS**, Zhang YL. Progress in research of colorectal cancer in China. *Shijie Huaren Xiaohua Zazhi* 2001; **9**: 489-494

2 **Colonna M,** Grosclaude P, Launoy G, Tretarre B, Arveux P, Raverdy N, Benhamiche AM, Herbert C, Faivre J. Estimation of colorectal cancer prevalence in France. *Eur J Cancer* 2001; **37**: 93-96

3 **Greenlee RT**, Murray T, Bolden S, Wingo PA. Cancer statistics, 2000. *CA Cancer J Clin* 2000; **50**: 7-33

4 **Greenlee RT**, Hill-Harmon MB, Murray T, Thun M. *Cancer statistics*, 2001. CA Cancer J Clin 2001; **51**: 15-36

5 **Konturek PC**, Bielanski W, Konturek SJ, Hartwich A, Pierzchalski P, Gonciarz M, Marlicz K, Starzynska T, Zuchowicz M, Darasz Z, Götze JP, Rehfeld JF, Hahn EG. Progastrin and cyclooxygenase-2 in colorectal cancer. *Dig Dis Sci* 2002; **47**: 1984-1991

6 **Saga T**, Tamaki N, Itoi K, Yamazaki T, Endo K, Watanabe G, Maruno H, Machinami R, Koizumi K, Ichikawa T, Takami H, Ishibashi M, Kubo A, Kusakabe K, Hirata Y, Murata Y, Miyachi Y, Tsubuku M, Sakahara H, Katada K, Tonami N,

Yamamoto K, Konishi J, Imamura M, Doi R, Shimatsu A, Noguchi S, Hasegawa Y, Ishikawa O, Watanabe Y, Nakajo M. Phase III additional clinical study of 111In-pentetreotide (MP-1727): diagnosis of gastrointestinal hormone producing tumors based on the presence of somatostatin receptors. *Kaku Igaku* 2003; **40**: 185-203

7 **Sadji-Ouatas Z**, Lasfer M, Julien S, Feldmann G, Reyl-Desmars F. Doxorubicin and octreotide induce a 40 kDa breakdown product of p53 in human hepatoma and tumoral colon cell lines. *Biochem J* 2002; **364**: 881-885

8 **Watson SA**, Morris TM, McWilliams DF, Harris J, Evans S, Smith A, Clarke PA. Potential role of endocrine gastrin in the colonic adenoma carcinoma sequence. *Br J Cancer* 2002; **87**: 567-573

9 **Wu P**, Tu JS, Riu J, Hang H, Hang WB, Yuan P. To study the correlation between expression of gastrin, somatostatin and cell proliferation, apoptosis in colorectal carcinoma. *Zhonghua Shiyan Waike Zaizhi* 2003; **20**: 947

10 **Fromowitz FB**, Viola MV, Chao S, Oravez S, Mishriki Y, Finkel G, Grimson R, Lundy J. ras p21 expression in the progression of breast cancer. *Hum Pathol* 1987; **18**: 1268-1275

11 **Hartwell LH,** Kastan MB. Cell cycle control and cancer. *Science* 1994; **266**: 1821-1828

12 **Arisi E,** Pruneri G, Carboni N, Sambataro G, Pignataro L. Prognostic significance of P27 and cyclin D1 co-expression in laryngeal squamous cell carcinoma: possible target for novel therapeutic strategies. *J Chemother* 2004; **16 Suppl 5**: 3-6

13 **Kudo Y,** Kitajima S, Ogawa I, Miyauchi M, Takata T. Down-regulation of Cdk inhibitor p27 in oral squamous cell carcinoma. *Oral Oncol* 2005; **41**: 105-116

14 **Hanahan D,** Weinberg RA. The hallmarks of cancer. *Cell* 2000; **100**: 57-70

15 **Sandal T.** Molecular aspects of the mammalian cell cycle and cancer. *Oncologist* 2002; **7**: 73-81

16 **Elledge SJ**. Cell cycle checkpoints: preventing an identity crisis. *Science* 1996; **274**: 1664-1672

17 **Morgan DO**. Principles of CDK regulation. *Nature* 1995; **374**:131-134

18 **Sherr CJ.** Cancer cell cycles. *Science* 1996; **274**: 1672-1677

19 **Sherr CJ**. The Pezcoller lecture: cancer cell cycles revisited. *Cancer Res* 2000; **60**: 3689-3695

20 **Tetsu O,** McCormick F. Proliferation of cancer cells despite CDK2 inhibition. Cancer Cell 2003; **3**: 233-245

21 **Yu Q**, Geng Y, Sicinski P. Specific protection against breast cancers by cyclin D1 ablation. *Nature* 2001; **411**: 1017-1021

22 **Managlia EZ**, Landay A, Al-Harthi L. Interleukin-7 signalling is sufficient to phenotypically and functionally prime human CD4 naive T cells. *Immunology* 2005; **114:** 322-335

23 **Yang M,** Huang HL, Zhu BY, Tuo QH, Liao DF. Onychin inhibits proliferation of vascular smooth muscle cells by regulating cell cycle. *Acta Pharmacol Sin* 2005; **26:** 205-211

24 **Li B,** DiCicco-Bloom E. Basic fibroblast growth factor exhibits dual and rapid regulation of cyclin D1 and p27 to stimulate proliferation of rat cerebral cortical precursors. *Dev Neurosci* 2004;26:197-207

25 **Zhang W,** Bergamaschi D, Jin B, Lu X. Posttranslational modifications of p27kip1 determine its binding specificity to different cyclins and cyclin-dependent kinases in vivo. *Blood* 2005; **105**: 3691-3698

26 **Planas-Silva MD**, Weinberg RA. The restriction point and control of cell proliferation. *Curr Opin Cell Biol* 1997; **9**: 768-772

27 **Huang X,** Di Liberto M, Cunningham AF, Kang L, Cheng S, Ely S, Liou HC, Maclennan IC, Chen-Kiang S. Homeostatic cell-cycle control by BLyS: Induction of cell-cycle entry but not G1/S transition in opposition to p18INK4c and p27Kip1. *Proc Natl Acad Sci USA* 2004; **101**: 17789-17794

28 **King KL,** Cidlowski JA. Cell cycle regulation and apoptosis. *Annu Rev Physiol* 1998; **60**: 601-617

29 **Senderowicz AM.** Small molecule modulators of cyclin-dependent kinases for cancer therapy. *Oncogene* 2000; **19**: 6600-6606

30 **Owa T**, Yoshino H, Yoshimatsu K, Nagasu T. Cell cycle regulation in the G_1 phase: a promising target for the development of new chemotherapeutic anticancer agents. *Curr Med Chem* 2001; **8**: 1487-1503

31 **Sereti E**, Gavriil A, Agnantis N, Golematis VC, Voloudakis-Baltatzis IE. Immunoelectron study of somatostatin, gastrin and glucagon in human colorectal adenocarcinomas and liver metastases. *Anticancer Res* 2002; **22**: 2117-2123

32 **Larsson LI**. Developmental biology of gastrin and somatostatin cells in the antropyloric mucosa of the stomach. *Microsc Res Tech* 2000; **48**: 272-281

33 **Portela-Gomes GM**, Albuquerque JP, Ferra MA. Serotonin and gastrin cells in rat gastrointestinal tract after thyroparathyroidectomy and induced hyperthyroidism. *Dig Dis Sci* 2000; **45**: 730-735

34 **Glover SC**, Tretiakova MS, Carroll RE, Benya RV. Increased frequency of gastrin-releasing peptide receptor gene mutations during colon-adenocarcinoma progression. *Mol Carcinog* 2003; **37**: 5-15

35 **Carroll RE,** Matkowskyj KA, Tretiakova MS, Battey JF, Benya RV. Gastrin-releasing peptide is a mitogen and a morphogen in murine colon cancer. *Cell Growth Differ* 2000; **11**: 385-393

36 **Tejeda M**, Gaal D, Barna K, Csuka O, Kéri G. The antitumor activity of the somatostatin structural derivative (TT-232) on different human tumor xenografts. *Anticancer Res* 2003; **23**: 4061-4066

37 **Yu HG**, Schrader H, Otte JM, Schmidt WE, Schmitz F. Rapid tyrosine phosphorylation of focal adhesion kinase, paxillin, and p130Cas by gastrin in human colon cancer cells. *Biochem Pharmacol* 2004; **67**: 135-146

38 **Wu H**, Rao GN, Dai B, Singh P. Autocrine gastrins in colon cancer cells Up-regulate cytochrome c oxidase Vb and down-regulate efflux of cytochrome c and activation of caspase-3. *J Biol Chem* 2000; **275**: 32491-32498

39 **Swatek J**, Chibowski D. Endocrine cells in colorectal carcinomas. Immunohistochemical study. *Pol J Pathol* 2000; **51**: 127-136

40 **Song DH,** Rana B, Wolfe JR, Crimmins G, Choi C, Albanese C, Wang TC, Pestell RG, Wolfe MM. Gastrin-induced gastric adenocarcinoma growth is mediated through cyclin D1. *Am J Physiol Gastrointest Liver Physiol* 2003: **285**: G217- G 222

41 **Yao M,** Song DH, Rana B, Wolfe MM. COX-2 selective inhibition reverses the trophic properties of gastrin in colorectal cancer. *Br J Cancer* 2002; **87**: 574-579

42 **Shen K,** He S, He Y. Effects of proglumide, a gastrin receptor antagonist, on human large intestine carcinoma SW480 cell line. *Chin Med J (Engl)* 1998; **111**: 1075-1078

43 **Pradeep A,** Sharma C, Sathyanarayana P, Albanese C, Fleming JV, Wang TC, Wolfe MM, Baker KM, Pestell RG, Rana B. Gastrin-mediated activation of cyclin D1 transcription involves beta-catenin and CREB pathways in gastric cancer cells. *Oncogene* 2004; **23**: 3689-3699

44 **Lefranc F,** Sadeghi N, Metens T, Brotchi J, Salmon I, Kiss R. Characterization of gastrin-induced cytostatic effect on cell proliferation in experimental malignant gliomas. *Neurosurgery* 2003; **52**: 881-890; discussion 890-891

45 **Zatelli MC**, Piccin D, Tagliati F, Ambrosio MR, Margutti A, Padovani R, Scanarini M, Culler MD, degli Uberti EC. Somatostatin receptor subtype 1 selective activation in human growth hormone (GH)- and prolactin (PRL)-secreting pituitary adenomas: effects on cell viability, GH, and PRL secretion. *J Clin Endocrinol Metab* 2003; **88**: 2797-2802

46 **Guillermet J,** Saint-Laurent N, Rochaix P, Cuvillier O, Levade T, Schally AV, Pradayrol L, Buscail L, Susini C, Bousquet C. Somatostatin receptor subtype 2 sensitizes human pancreatic cancer cells to death ligand-induced apoptosis. *Proc Natl Acad Sci USA* 2003; **100:** 155-160

47 **Faiss S,** Pape UF, Böhmig M, Dörffel Y, Mansmann U, Golder W, Riecken EO, Wiedenmann B.Prospective, randomized, multicenter trial on the antiproliferative effect of lanreotide, interferon alfa, and their combination for therapy of

metastatic neuroendocrine gastroenteropancreatic tumors--the International Lanreotide and Interferon Alfa Study Group. *J Clin Oncol* 2003; **21**: 2689-2696

48 **Benali N,** Ferjoux G, Puente E, Buscail L, Susini C. Somatostatin receptors. *Digestion* 2000; **62 Suppl 1**: 27-32

49 **Hortala M,** Ferjoux G, Estival A, Bertrand C, Schulz S, Pradayrol L, Susini C, Clemente F. Inhibitory role of the somatostatin receptor SST2 on the intracrine-regulated cell proliferation induced by the 210-amino acid fibroblast growth factor-2 isoform: implication of JAK2. *J Biol Chem* 2003; **278**: 20574-20581

50 **Buscail L,** Vernejoul F, Faure P, Torrisani J, Susini C. Regulation of cell proliferation by somatostatin. *Ann Endocrinol (Paris)* 2002; **63(2 Pt 3)**: 2S13-2S18

51 **Puente E,** Saint-Laurent N, Torrisani J, Furet C, Schally AV, Vaysse N, Buscail L, Susini C. Transcriptional activation of mouse sst2 somatostatin receptor promoter by transforming growth factor-beta. Involvement of Smad4. *J Biol Chem* 2001; **276**: 13461-13468

52 **Ferjoux G,** Bousquet C, Cordelier P, Benali N, Lopez F, Rochaix P, Buscail L, Susini C. Signal transduction of somatostatin receptors negatively controlling cell proliferation. *J Physiol Paris* 2000; **94**: 205-210

53 **Pagès P,** Benali N, Saint-Laurent N, Estève JP, Schally AV, Tkaczuk J, Vaysse N, Susini C, Buscail L.sst2 somatostatin receptor mediates cell cycle arrest and induction of p27(Kip1). Evidence for the role of SHP-1. *J Biol Chem* 1999; **274**: 15186-15193

54 **Charland S,** Boucher MJ, Houde M, Rivard N. Somatostatin inhibits Akt phosphorylation and cell cycle entry, but not p42/p44 mitogen-activated protein (MAP) kinase activation in normal and tumoral pancreatic acinar cells. *Endocrinology* 2001; **142**: 121-128

55 **Zhao B,** Zhao H, Zhao N, Zhu XG. Cholangiocarcinoma cells express somatostatin receptor subtype 2 and respond to octreotide treatment. *J Hepatobiliary Pancreat Surg* 2002; **9**: 497-502

56 **Wu P,** Rui J, Xia XH, Yuan P, Ma Y, Zhou G. Expression of Gastrin, somatostatin and their specific power in colorectal carcinoma. *Zhonghua Shiyan Waike Zaizhi* 1998; **11**: 520-521

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