Regulatory effect and mechanism of gastrin and its antagonists on colorectal carcinoma

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Abstract

AIM To explore the effect and mechanism of gastrin and its agonists proglumide and somatostatin on colorectal carcinoma and their clinical significance.

METHODS A model of transplanted human colonic carcinoma was established from SW480 cell line in gymnomouse body. The volume and weight of transplanted carcinoma was observed under the effect of pentagastatin (PG), proglumide (PGL) and octapeptide somotostatin (SMS). The cAMP content of carcina noma cell was determined by radiommmunoassay and the DNA, protein content and cell cycle were determined by flow-cytometry. The amount of viable cells was determined by MTT colorimetric analysis, IP₃ content was determined by radioimmuno assay, Ca²⁺ concentration in cell by fluorometry and PKC activity by isotopic enzymolysis. The expression of gastrin, c-myc, c-fos and rasP21 in 48 case s of colorectal carcinoma tissue was detected by the immunocytochemistry SP method. Argyrophilia nucleolar organizer regions was determined with argyrophilia stain.

RESULTS The volume, weight, cAMP, DNA and protein content in carcinoma cell, cell amount and proliferation index of S and G₂M phase in PG group were all significantly higher than those of control group. When PG was at the concentration of 25 mg/L, the amount of viable cells, IP₃ content and Ca²⁺ concentration in cell and membrane PKC activity in PG group were significantly higher than those in control group; when PGL was at a concentration of 32 mg/L, they dropped to the lowest level in PG (25 mg/L) +PGL group, but without significant difference from the control group. The positive expression rate of gastrin, c-myc, c-fos and rasP21 in carcinoma tissue was 39.6%, 54.2%, 47.9% and 54.2% respectively and significantly higher than that in mucosa 3 cm and 6 cm adjacent to carcinoma tissue and normal colorectal mucosa. The positive expression rate of gastrin of highly-differentiated adenocarcinoma group was significantly higher than that of poorly-differentiated and mucinous adenocarcinoma groups. The AgNORs count of carcinoma tissue was significantly higher than that in mucosa 3 cm and 6 cm adjacent to carcinoma tissue and normal colorectal mucosa; and the positive expression of c-myc and c-fos and the AgNORs count in gastrin-positive group was significantly higher than those in gastrin-negative group.

CONCLUSION Pentagastrin has a promoting effect on the growth of transplanted human colonic carcinoma from SW480 cell line. PGL has no obvious effect on the growth of human colonic carcinoma SW480 cell line, but could inhibit the growth promoting effect of PG on transplanted carcinoma. Somatostatin can not only inhibit the growth of transplanted human colonic carcinoma from SW480 cell line directly but also depress the growth-promoting effect of gastrin on the transplanted carcinoma. Some colorectal carcinoma cells can produce and secrete gastrin through autocrine, highly-differentiated adenocarcinoma express the highest level gastrin. Endogenous gastrin can stimulate the cell division and proliferation of carcinoma cell and promote the growth of colorectal carcinoma regulating the expression of oncogene c-myc, c-fos. Our study has provided experimental basis for the adjuvant treatment using gastrin antagonist such as PGL, so somatostatin of patients with colorectal carcinoma.
INTRODUCTION
In recent years, some studies indicated that gastrin could promote the growth of some colorectal carcinomas, but gastrin antagonist such as PGL, somatostatin (SS) could inhibit the growth of those colorectal carcinomas. In order to explore the mechanism of the effect and the clinical significance of gastrin and its antagonists on colorectal carcinoma, we established the model of transplanted colonic carcinoma from SW480 cell line in gymnouse body, and observed the volume and weight of transplanted carcinoma, content of Ca2+, DNA, protein and cell cycle of carcinoma cell under the effect of pentagastrin (PG), proglumide (PGL) and octapeptide somatostatin (SMS 201-995, SMS). We also observed the effect of PG and PGL on the amount of viable cells, inositol 1, 4, 5-trisphosphate (IP3) content and Ca2+ concentration in cell and protein kinase C (PKC) activity of colonic carcinoma SW480 cell line cultured in vitro, and detected the expression of gastrin, cancer genes c-myc, c-fos and rasP21 in 48 cases of colorectal carcinoma tissue by the immunocytochemical SP method and determined the argyrophilia nucleolar organizer regions count with argyrophilia stain.

MATERIALS AND METHODS
Materials
Human colonic carcinoma SW480 cell line (Sloan Kettering Memorial Cancer Center, US A), BALB/C gymnouse (Experimental Animal Center of Third Military Medical University), pentagastrin (PG, Shanghai Lizhu Chemical Reagent Co.), octapeptide somatostatin (SMS 201-995, SMS, PHARMA, Swiss); proglumide (PGL, No.11 Pharmacentical Factory, Shanghai), RPMI1640 culture solution (GIBCO, USA), 3H-myo-inositol (Institute of Atomic Energy, Chinese Academy of Sciences), T-32P-ATP (Ya Hui Biology Engineer Co.), Fura-2/AM (Sigma, USA), cAMP radioimmuno noassay reagent kit (Department of Nuclear Medicine of Shanghai Second Medical University), monoclonal antibody to gastrin and SP kit (ZYMED Co., USA), monoclonal antibody for c-myc and rasP21, monoclonal antibody for c-fos (Santa Cruz Co., USA).

Experiment methods
Establishment of transplanted carcinoma model The human colonic carcinoma SW480 cell line was resuscitated conventionally. Cell lines with exuberant vitality were selected to form primary transplanted carcinoma, digested with 0.25% trypsin, centrifugated to wipe off the digestive solution, and adjust the cell concentration with RPMI1640 cultural solution to 5×10^6/mL. The living cell amount exceeded 99% by trypan-blue stain. Twelve gymnouse were ran domized into six groups. The cell line went through six passages, each time two gymnouse were inoculated. After 0.2 mL of carcinoma cell solution was inoculated to the back of the neck of gymnouse, the gymnouse were raised in clean room. When the diameter of the carcinoma reached 1.5 cm to 2.2 cm, the gymnouse were killed by severing the cervical vertebra, and the carcinoma mass was stiped bacteria-freely. After preserving specimen for histological and electron microscopical examination, the mass was smashed, ground into cell suspension with RPMI1640 cultural solution, adjusted the cell concentration to 5×10^6/mL, the next passage was started with 0.2 mL of the cell suspension.

Experimental animal grouping After undergoing passages stably-growing human colonic transplanted carcinoma cells were inoculated into thirty gymnouse. They were randomized into six groups and injected with the experimental drugs subcutaneously the next day, two times per day, for 35 days. The gymnouse were killed 24 hours after the last injection. Control group: 0.4 mL normal saline/mouse. PG group: 4 µg PG/mouse. PGL group: 6 µg PGL/mouse. SMS group: 6 µg SMS/mouse. PG+PGL group: 4 µg PG and 10 mg PGL/mouse. PG+SMS group: 4 µg PG and 6 µg SMS/mouse.

Volume and weight of transplanted carcinoma The long diameter and short diameter were measured at the same time every sixth days after the inoculation, and the measurement lasted six weeks. The volume was calculated by the formula: V = 1/2a2b. The weight of the mass was then determined after decontamination of the non tumor tissue such as blood and fat tissue.

Flow-cytometry Fifteen g transplanted carcinoma tissue was cut and made into single-cell suspension, adjusted to a cell concentration of 1×10^6/L, and fixed with 700 g/L alcohol at 4 °C. The DNA was stained with propidium iodide to redish flourescence and the protein stained with fluorescein iso thiocyanate to green flourescence. Thirty minutes later, the suspension was analyzed on instrument at room temperature. According to Barlogie cell cycle analysis method, the cells were divided into three parts: G0/G1 phase, S phase and G-2M phase. Proliferation index (PI) of the cell was calculated as: PI = (S+G2M)/(G0/G1+S+G2M)×100%.

MTT colorimetric analysis Suspend the large intestine carcinoma SW480 cells in logarithmic
growth stage in 100g/L bovine serum culture solution to a concentration of 5×10^{-3}/L. The suspension was inoculated in a 96-well plate (100 µL/well) and cultivated for 48 h. After the supernatant was wiped off, 100 µL of 5 g/L bovine serum culture solution was added to each well and the first well as zero control, the second one as cell control, which were added with 100 µL of 5 g/L bovine serum culture solution respectively. From the third one on, 100 µL of different drugs with different concentrations were added to each well as follows and repeated 8 times: PG group: the concentration was 6.25, 12.50, 25.00, 50.00 and 100.00 mg/L, respectively. PG (25 mg/L)+PGL group: the concentration of PGL was 8.00, 16.00, 32.00, 64.00 and 128.00 mg/L. They were cultivated in 37 °C, 50 g/L CO₂ incubator for 18 hours, then add LiCl solution, 2 hours later, add 100 µL of 5 g/L MTT solution 6 hours before terminating the culture and 100 µL of 200 g/LSDS solution was added at the termination, kept overnight in the incubator, and the absorptivity (A value) at the 570 nm wavelength was determined on the instrument the next day.

**Determination of IP₃ content in carcinoma cell** Add 15 mCi/L of [³H]-inositol into the suspension of SW480 cell line, cultivate them in 37 °C, 50 g/L CO₂ incubator for 18 hours, then add LiCl solution, 2 hours later, add 100 µL different drugs with different concentrations to each well and cultivate 1 minute as follows: PG group: the concentration was 6.25, 12.50, 25.00, 50.00 and 100.00 mg/L, respectively; PG (25 mg/L)+PGL group: the concentration of PGL is 16.00, 32.00 and 64.00 mg/L. Separate IP₃ from the suspension with anion change separation column according to the method described in reference [3,4], then determine its CPM value with hydroflicker.

**Determination of PKC activity** Determine the PKC activity in human colonic carcinoma SW480 cell line under the effect of PG and PGL according to the method described in reference [5] with isotopic enzymolysis. The grouping and concentration of PG and PGL were the same with IP₃ determination.

**Determination of Ca²⁺ concentration** Determine the Ca²⁺ concentration in human colonic carcinoma SW480 cell line under the effect of PG and PGL according to the method described in reference [6] with flurometry. The grouping and concentration of PG and PGL were the same with IP₃ determination.

**Specimen** Forty-eight cases of radical resection of colorectal carcinomas collected from August 1996 to April 1997 in our department were chosen as experimental materials. Ten cases of normal colorectal mucosa served as control group, including 3 cases of inner prolapse of rectum, 2 of volvulus of sigmoid and 5 of sudden death. All the specimens were confirmed by pathological examination. Fresh colorectal carcinoma tissue (3 g) and mucosas 3 cm and 6 cm adjacent to carcinoma were cut respectively, fixed with 40 g/L pa raformaldehyde, dehydrated and embedded with paraffin.

**Immunohistochemistry method** Immunohistochemistry staining of gastrin, c-myc, c-fos and rasP21 was carried out by SP method, referring to the illustration of reagent kit. The first antibody dilution of gastrin was 1:50, while the dilution of c-myc, c-fos and rasP21 was all 1:100. Microwv e was used to repair the protein of c-myc, c-fos and rasP21. Normal mucosa of antrum was taken as positive control for gastrin, positively stained tissue of hepatic carcinoma served as positive control for c-myc, c-fos and rasP21. PBS solution replacing the first antibody was taken as negative control. Cells containing brown-yellow pellets were considered as positive cells.

**Argyrophilia nucleolar organizer regions count (AgNORs)** Argyrophia staining was carried out according to Hu PH’s modified method [7] and observed under ×100 oil microscope. The nucleus background appeared light yellow while the AgNORs granule appeared palm-black. The amount of AgNORs granule of 50-200 cells and the mean value were calculated.

**Statistics** All the data was expressed as mean±standard deviation (±s), and analysed with t test or one-way analysis of variance; the differences between the rates of different groups were analysed by χ² test.

**RESULTS**

**Model of transplanted human colonic carcinoma in gymnomouse**

The inoculation of transplanted carcinoma was 100% successful, no gymnomouse died. The inoculation period was 6-8 days, the speed of growth became stable till the sixth generation. At the end of the 5th week, the long diameter of the mass reached 1.6 cm-2.0 cm. It was elliptical in shape and smooth on surf ace in the early stage; while in the advanced stage, the shape became irregular and the surface became nodal. The histological H.E. stain and ultrastructure of the transplanted carcinoma had the same pathological feature of human colonic carcinoma.

**Effect of PG, PGL and SMS on the transplanted...**
human colonic carcinoma in gymnomouse

All the gymnomic bearing the transplanted carcinoma survived at the end of the experiment. The volume, weight, cAMP, DNA and protein content in carcinoma cell, cell amount and proliferation index of S and G,M phase in PG group were significantly higher than those of control group (P<0.05-0.01), markedly lower in PGL and PG+PGL group in PG group (P<0.05-0.01), yet there was no statistical difference between PG, PG+PGL groups and control group (P>0.05); and markedly lower in SMS and SMS+PG group than in PG group and control group (P<0.01). The cell amount of G0/G1 phase in PG group was obviously lower than in control group (P<0.01), markedly higher in PGL and PG+PGL group than in PG group (P<0.01), without statistical difference between PGL, PG+PGL groups and control group (P>0.05); markedly higher in SMS and SMS+PG group than in PG group and control group (P<0.01, Tables 1-3).

Table 1 Effect of PG, PGL and SMS on the volume and weight of transplanted carcinoma (X±s, n = 5)

| Group          | Volume (mm³) | Weight (g) |
|----------------|--------------|------------|
| Control        | 1766±36      | 3.04±0.13  |
| PG             | 1926±98c     | 3.37±0.21a |
| PGL            | 1750±53a     | 2.98±0.16  |
| SMS            | 210±13b      | 0.90±0.14a |
| PG+PGL         | 1708±59b     | 2.91±0.23a |
| PG+SMS         | 224±19d      | 0.95±0.12a |

Table 2 Effect of PG, PGL and SMS on cAMP, DNA and protein content (X±s, n = 5)

| Group          | CAMP(pmol/mL) | DNA(dalton) | Proteint(dalton) |
|----------------|--------------|-------------|-----------------|
| Control        | 2.74±0.14    | 947±16      | 364±12          |
| PG             | 3.18±0.23b   | 1004±17b    | 675±18b         |
| PGL            | 2.72±0.15a   | 940±21      | 356±97          |
| SMS            | 1.87±0.14c   | 68±13c      | 27±11d          |
| PG+PGL         | 2.82±0.17c   | 940±25      | 369±14c         |
| PG+SMS         | 1.86±0.17c   | 687±21      | 274±13cd        |

Table 3 Effect of PG, PGL and SMS on cell cycle and proliferation index (PI)

| Group          | G0/G1 (%) | S (%) | G2M (%) | PI (%) |
|----------------|-----------|-------|---------|--------|
| Control        | 64.9±2.72 | 18.2±1.20 | 16.8±4.25 | 35.0±8±1.72 |
| PG             | 59.2±2.18 | 20.1±1.00 | 20.6±2.05a | 40.7±2.81a |
| PGL            | 67.18±2.23 | 18.0±4.13 | 14.7±1.09c | 32.8±2.27c |
| SMS            | 80.9±2.29c | 14.9±1.6d | 5.0±1.61d | 19.9±2.39d |
| PG+PGL         | 67.76±2.41 | 18.1±4.10 | 14.0±4.06d | 32.2±4.24a |
| PG+SMS         | 80.26±2.73 | 15.2±2.84d | 4.5±2.25a | 19.2±2.73a |

Effect of PG on the amount of viable cells (A value), IP3 content (CPM) and Ca2+ concentration in cell and membrane PKC activity of SW480 cell line

When PG was at the concentration of 12.5 mg/L, the amount of viable cells, IP3 content and Ca2+ concentration in the cells of PG group were markedly higher than those in control group (P<0.05) while it was at the concentration of 25 mg/L, they all reached the highest value, but plasma PKC activity decreased, and all had statistical difference from those of control group (P<0.05-0.01). When PG concentration exceeded 50 mg/L, these items did not continue to increase and plasma PKC activity did not continue to decrease, but they were all statistically different from those of control group (P<0.05-0.01, Table 4).

Effect of PG+PGL on the amount of viable cells (A value), IP3 content (CPM) and Ca2+ concentration in cell and membrane PKC activity of SW480 cell line

When PGL was at the concentration of 8 mg/L, the amount of viable cells (A value) of PG (25 mg/L)+PGL group was markedly smaller than that of PG group (P<0.01), when at the concentration of 16 mg/L, the amount of viable cells, IP3 content and Ca2+ concentration in cell and membrane PKC activity of PG+PGL group decreased and plasma PKC activity increased, all being statistically different from those of control group (P<0.05-0.01). At the concentration of 32 mL/L, they decreased to the lowest value and were markedly lower than those in PG group (P<0.05-0.01), and did not differ significantly from those of the control group (P>0.05). At the concentration of 64 mL/L, these items in PG+PGL group did not continue to decrease, but they all had statistical difference from those of PG group (P<0.05-0.01, Table 5).

Expression of gastric, c-myc, c-fos and rasP21 and AgNORs count in carcinoma tissue

The positive expression rate of gastrin, c-myc, c-fos and rasP21 in 48 cases of f carcinoma tissue was 39.6%, 54.2%, 479% and 54.2% respectively and significantly higher than that in mucosa 3 cm and 6 cm adjacent to carcinoma tissue and normal colorectal mucosa (P<0.01). The positive expression rate of gastrin of highly-differentiated adenocarcinoma was significantly higher than that of poorly-differentiated mucinous adenocarcinoma (P<0.05), there was no statistical difference in the positive expression rate of c-myc, c-fos and rasP21 between groups of different pathological types (P>0.05). The AgNORs counts of carcinoma tissue was significantly higher than that in mucosa 3 cm and 6 cm adjacent to carcinoma tissue and normal colorectal mucosa (P<0.01); and the count of mucosa 3 cm adjacent to carcinoma...
tissue was significantly higher than that in mucosa 6cm adjacent to carcinoma tissue and normal colorectal mucosa (P<0.05, Tables 6-7).

**Relationship between gastrin and the expression of c-myc, c-fos, rasP21 and AgNORs count in carcinoma tissue**

The positive expression rate of c-myc and c-fos and the AgNORs count in gastrin-positive group was significantly higher than those in gastrin-negative group (P < 0.05 - 0.01), while the positive expression rate of rasP21 in gastrin-positive group was not different from that in gastrin-negative group (P > 0.05, Table 8).

**Table 4** Effect of PG on VCC, IP₃, [Ca²⁺] and PKC activity (pmol/min per mg protein) (±s, n = 5)

| Group (mg/L) | VCC (A)     | IP₃ (CPM)   | [Ca²⁺]i (nM) | Plasma PKC | Membrane PKC |
|-------------|-------------|-------------|--------------|------------|--------------|
| Control     | 1.55±0.009  | 8.83±1.33   | 26.36±2.91   | 2.39±1.30  | 1.07±0.28    |
| PG 6.25     | 1.55±0.005  | 10.16±1.33  | 30.16±2.16   | 2.36±0.84  | 1.09±0.16    |
| 12.50       | 1.56±0.010a | 11.18±0.75a | 50.69±2.30a  | 2.30±0.45a | 1.15±0.15a   |
| 25.00       | 1.58±0.011b | 16.17±0.75b | 101.44±2.49b | 0.91±0.34b | 2.65±1.21b   |
| 50.00       | 1.57±0.008b | 14.10±1.60b | 70.63±4.17b  | 0.92±0.26b | 2.65±0.60b   |
| 100.00      | 1.57±0.010b | 13.00±2.53b | 62.59±2.59b  | 0.91±0.14b | 2.66±0.68b   |

*P<0.05, bP<0.01 vs control group.

**Table 5** Effect of PG+PGL on VCC, IP₃, [Ca²⁺]i and PKC activity (pmol/min per mg protein) (±s, n = 5)

| Group | PG (mg/L) | PGL (mg/L) | VCC (A) | IP₃ (CPM) | [Ca²⁺]i (nM) | Plasma PKC | Membrane PKC |
|-------|-----------|------------|---------|-----------|--------------|------------|--------------|
| Control | 0.00   | 0.00 | 1.55±0.009 | 8.83±1.33 | 26.36±2.91 | 2.39±1.30 | 1.07±0.28 |
| PG 25.00 | 25.00 | 0.00 | 1.58±0.011b | 16.17±0.75b | 101.44±2.49b | 0.91±0.34b | 2.65±0.21b |
| PG+PGL 25.00 | 25.00 | 8.00 | 1.55±0.016d | 9.17±1.47d | 32.63±2.86d | 2.33±0.29d | 1.05±0.09d |
| 25.00  | 25.00 | 16.00 | 1.55±0.008b | 9.00±1.58d | 31.79±4.41d | 2.30±0.61d | 1.05±0.20d |
| 25.00  | 25.00 | 32.00 | 1.54±0.011d | 9.33±1.97d | 32.45±2.46d | 2.32±0.17d | 1.09±0.12d |
| 25.00  | 25.00 | 64.00 | 1.54±0.011d | 9.00±1.58d | 31.79±4.41d | 2.30±0.61d | 1.05±0.20d |
| 25.00  | 25.00 | 128.00 | 1.54±0.014d | 9.33±1.97d | 32.45±2.46d | 2.32±0.17d | 1.09±0.12d |

*P<0.05, bP<0.01 vs control group; aP<0.05, dP<0.01 vs PG group.

**Table 6** Expression of gastrin, c-myc, c-fos, rasP21 and AgNORs

| Group                  | n | gastrin positive (%) | c-myc positive (%) | c-fos positive (%) | rasP21 positive (%) | AgNORs |
|------------------------|---|---------------------|--------------------|-------------------|---------------------|--------|
| Carcinoma tissue       | 48| 19(39.6)b           | 26(54.2)b          | 23(47.9)b         | 26(54.2)b           | 7.10±1.48b |
| 3cm mucosa             | 48| 2(4.2)              | 12(25.0)           | 9(18.8)           | 10(20.8)            | 3.65±1.04c |
| 6cm mucosa             | 48| 0(0)                | 7(14.6)            | 4(803)            | 6(12.5)             | 2.88±0.73 |
| Normal                 | 10| 0(0)                | 1(10.0)            | 1(10.0)           | 2(20.0)             | 2.85±0.60 |

*P<0.01 vs 3cm, 6cm and normal mucosa group. aP<0.05 vs 6cm and normal mucosa group.

**Table 7** Expression of gastrin, c-myc, c-fos, rasP21 and AgNORs in different pathological carcinoma tissue

| Group                    | n | Gastrin positive (%) | c-myc positive (%) | c-fos positive (%) | rasP21 positive (%) | AgNORs  |
|--------------------------|---|---------------------|--------------------|-------------------|--------------------|---------|
| Highly-differentiated    | 26| 14(53.9)a           | 15(57.7)           | 13(50.0)          | 15(57.7)           | 7.12±1.80 |
| Moderately-differentiated| 12| 4(33.3)             | 5(41.7)            | 7(58.3)           | 6(50.0)            | 7.25±0.86 |
| Poorly- and mucinous     | 10| 1(10.0)             | 6(60.0)            | 3(30.0)           | 5(50.0)            | 7.11±1.20 |

*P<0.05 vs poorly-differentiated and mucinous group.

**Table 8** Relation between gastrin and c-myc, c-fos, rasP21 and AgNO Rs

| Group                  | n | c-myc positive (%) | c-fos positive (%) | rasP21 positive (%) | AgNORs  |
|------------------------|---|--------------------|--------------------|---------------------|---------|
| Gastrin-positive       | 19| 15(78.9)b          | 14(73.7)b          | 11(57.9)           | 7.84±1.30 |
| Gastrin-negative       | 29| 11(37.9)           | 9(31.0)            | 15(51.7)           | 6.22±1.40 |

*P<0.05, bP<0.01 vs gastrin-negative group.
DISCUSSION

The main physiological function of gastrin is to stimulate the secretion of gastric acid and nourish the gastrointestinal mucosa. In recent years, it has been found that some gastrointestinal carcinomas could express gastrin gene and there existed gastrin receptor on the carcinoma cell membrane, and gastrin could stimulate the growth of gastrin carcinoma. So, more and more researchers focus their interest on the relationship between gastrin and colonic carcinoma.

The transplanted human colonic carcinoma model from SW480 cell line in gynnomouse established by us remained the biological characteristics of colonic carcinoma. After subcutaneous injection of 8 mg pentagastrin per day for 35 days, the volume and weight of transplanted carcinoma, the cAMP content in carcinoma cell were significantly higher than those of control group, indicating that gastrin promoted the proliferation of transplanted colonic carcinoma. Ishizuka reported that the combination of gastrin and gastrin could promote the growth of gastrin carcinoma cell, while the decreased expression of cAMP-dependent protein kinase. The increased difference depended on the quality and quantity of cAMP or protein kinase C pathway to regulate the cell growth and differentiation. Mauss found that the promoting effect of gastrin on colonic carcinoma cell was selective, marginapeptide gastrin stimulated the growth of HT29, LoVo, CoLo32 cell line, but inhibited the growth of T84, HCT116 cell lines. The difference depended on the quality and quantity of cAMP-dependent protein kinase. The increased expression of Type I cAMP protein kinase could promote the differentiation and growth of carcinoma cells, while the decreased expression of Type IcAMP protein kinase or the increased expression of Type II cAMP protein kinase could inhibit the differentiation and growth of carcinoma cells. Baldwin reported that gastrin could promote the growth of 50 percent of the transplanted colonic carcinoma in vivo. We also determined the DNA, protein content and cell cycle by flow-cytometry, and found that DNA and protein content in carcinoma cell and the cell amount and proliferation index of S and G2M phase of PG group were significantly higher than those of control group, while the cell amount of G0/G1 phase of PG group was significantly lower than that of control group. It indicated that the mechanism for the promoting effect of gastrin on the cell division and proliferation of the human colonic carcinoma SW480 cell line might be that gastrin could promote the synthesis of cAMP, DNA and protein in carcinoma cells, the cell growth from G0/G1 to S and G2M phase, and regulate the cell cycle of colonic carcinoma cells after receptor.

Venter firstly found that there existed high appetency gastrin receptor on the membrane of human colonic carcinoma cell line LoVo and MC-26, both the nutritious effect of gastrin on the mucosa of normal large intestine and the growth-promoting effect of gastrin on large intestine carcinoma were realized through the combination of gastrin and its receptor on the membrane. We found by MTT colorimetric analysis that pentagastrin could promote the increase of viable cell count of colonic carcinoma SW480 cell line and the effect had a dose-effect dependent relationship with the concentration to some extent, along with the increase of dosage of pentagastrin, its growth-promoting effect did not continue to increase, but inclined to a stable level. This finding was consistent with the receptor theory, i.e., the receptor had saturation and indirectly proved that the growth promoting effect of gastrin on large intestine carcinoma was intermediated by gastrin receptor.

Some researches indicate that gastrin is a kind of autocrine growth-promoting factor. Hoosein et al. found that after the colonic carcinoma HCT1 16 and CBS cell line were cultured for 72 hours, the concentration of gastrin was 10.15 pg/10^6 cell. Finley et al. found by the immunocytochemical method that at least 50% of the colorectal carcinoma cells expressed gastrin. In our experiment, 19 of 48 cases of colorectal carcinoma expressed gastrin, with a positive rate of 39.6%, which was significantly higher than that in mucosa adjacent to carcinoma and normal mucosa, the expression rate of gastrin in highly-differentiated group was the highest (53.9%), all these indicated that some colorectal carcinomas could produce gastrin in an autocrine manner. Translational processing of gastrin mRNA to precursor forms of gastrin needs the participation of multiple enzymes and cofactors to produce mature gastrin finally. Therefore, the sound processing enzymes such as peptidylglycine α-amidating monoxygenase and cofactors in well differentiated carcinoma cells may contribu te to the production of mature gastrin, while the lack of other cofactors or enzymes in poorly-differentiated carcinoma cells may contribute to incomplete processing of precursor forms of gastrin and the deficiency of mature gastrin[17]. So, the gastrin expression of highly-differentiated adenocarcinoma was obviously higher than that of poorly-differentiated one.

We firstly applied the AgNORs technique to the clinical study of the growth-promoting effect of gastrin on large intestine carcinoma. The AgNORs count could mirror the structure and function of nucleolus, the transcription activity of rRNA and the cell proliferation. The AgNORs count of
colorectal carcinoma in our study was significantly higher than that of mucosa adjacent to carcinoma and normal mucosa, while the count of mucosa 3cm adjacent to carcinoma was higher than that of mucosa 6cm adjacent to carcinoma and normal mucosa. It indicated that the DNA in carcinoma cell and mucosa 3cm adjacent to carcinoma was in a disorder state, the regulation of cell proliferation was uncontrollable. We also found that the AgNORs count of gastrin-positive group was significantly higher than that in gastrin-negative group, indicating that endogenous gastrin had growth-promoting effect on some kinds of colorectal carcinomas.

Oncogene c-myc and c-fos is a kind of effect protein of the karyomitosis signal, which can trigger and regulate the transcription of the genes related with proliferation, besides, c-fos can also regulate its own gene expression with a positive feedback and promote the mitosis and proliferation of the cells[19]. Oncogene ras, a kind of GPT protein located at cell plasma and membrane, participates in the signal transduction regulation of various growth factor receptors. Once being mutant, ras oncogene will be continuously activated and obviously promote the mitosis of the cells[19]. Our results revealed that the positive expression rate of gastrin, c-myc, c-fos and rasP21 in carcinoma tissue was significantly higher than that in mucosa adjacent to carcinoma tissue and normal colorectal mucosa, the positive expression rate of c-myc and c-fos in gastrin-positive group was significantly higher than that in gastrin-negative group. It indicated that the growth-promoting effect of gastrin on colorectal carcinoma may be correlated with the activation of oncogenes. Wang et al[20] found that administration of gastrin resulted in the rapid appearance of c-myc mRNA in IEC-6 cells, the maximum increase in c-myc mRNA levels was 7.5-fold that of the normal value. Andrea et al[21,22] reported that gastrin had a promoting effect on the growth of AR4-2J cell line, and could induce the increase of c-fos mRNA content; after having combined with its receptor, gastrin triggered a series of phosphorylation in PKC signal pathway and induced the activation of extracellular signal regulatory kinase ERK 2, the kinase increased the transcription activity of EIK-1, then enlarged the expression of c-fos gene and stimulated the cell proliferation. Seva et al[23] reported that gastrin stimulated MAP kinase activation in a dose- and time dependent manner, rasP21 may link the MAP kinase pathway to gastrin receptors to trigger the activation.

Phosphatidylinositol signal pathway played an important role in the biological transmembrane transduction, which was intimately correlated with cell proliferation and tumorgenesis[24]. Phosphatidylinositol-4, 5-bisphosphate (PIP-2) was the direct precursor of second messengers IP3 and PG, and it functioned through IP3-Ca2+ pathway and DG-PKC pathway. The ascendance of dissociate Ca2+ concentration was an important mitosis promoting signal and Ca2+ correlated with the genesis and growth of many kinds of carcinomas. Protein kinase C was a kind of important kinase in the phosphatidylinositol signal path way, it existed in plasma of static cells by a non activation form, and once it was activated, it moved to cell membrane. The changes of activity were intimately correlated with cell proliferation. In our study, after we applied pentagastrin to colonic carcinoma SW480 cell line, the VCC, intracellular IP3 content and Ca2+ concentration and membrane PKC activity all ascended and were dose-d ependant obviously. But when pentagastrin and antagonist of gastrin were taken together, they remained changed, demonstrating that the growth-promoting effect of pentagastrin on human colonic carcinoma SW480 cell line was correlated with phosphatidylinositol signal pathway and it was through its receptor intermediate function in phosphatidylinositol signal pathway that proglumide antagonized the growth-promoting effect of gastrin on carcinoma cell. Ishizuka et al[10] suggested that the combination of gastrin and its receptor activated the membrane phospholipase and then hydrolyzed phosphatide to PI and DG, the latter then activated PKC, and finally Ca2+ was released and functioned.

All the findings above and our results indicated that some carcinoma cells produced gastrin by autocrine, after being intermediated by the second messenger in the cells, the expression of oncogene c-myc and c-fos was enlarged, thus promoting the proliferation of the carcinoma cells.

To block any link in the function procedure of gastrin could weaken or inhibit the growth-promoting effect of gastrin on large intestine carcinoma. There were three kinds of gastrin receptor antagonists: proglumide, somatostatin and prostaglandin (PG). Most of the interests were focused on proglumide, amide, the functional group of proglumide could particularly compete gastrin receptor with gastrin. In our research, 10mg proglumide was injected subcutaneously into transplanted carcinoma in gymnomic, 35 days later, compared with control group, there was no statistical difference in the volume, weight, intracellular cAM P, DNA, protein content and cell cycle of transplanted carcinoma in the PG group, but all the value above including cell amount of S and G 2M phase, proliferation index of PG and proglumide group were significantly lower than those of PG group, while the cell amount of G0/G1 phase was higher than that of PG group. All these
showed that proglumide had no effect on the growth of human colonic carcinoma SW480 cell line but could inhibit the growth-promoting effect of gastrin on transplanted carcinoma. Proglumide has been applied to the clinical practice. K aneyama et al\(^{[25]}\) reported that seven large intestine carcinoma patients with hepatic metastases were treated with proglumide (three times daily, each time 400mg) and 5’DFUR (800mg) for 2 years after the resection of the hepatic metastatic mass, at the same time, patients received chemotherapy by hepatic artery encheiresis and followed up for an average of 39 months. The relapse rate of the proglumide+chemotherapy group was 14% (1/7), while the rate of the chemot herapy alone group was 52% (14/26), so they drew a conclusion that the hormone therapy could effectively prevent the relapse after the resection of the hepatic metastatic mass. We used proglumide as an adjuvant drug to treat 25 colorectal patients having radical resection, besides administration of 400mg proglumide three times a day, MFA chemotherapy program was undertaken at the same time, while 25 patients as control group undertook MFA chemotherapy program alone. Follow-up results indicated that the 3-year survival rate of proglumide group (80%) was higher than that in control group (64%), the relapse or metastasis rate of proglumide group (12%) was lower than that in control group (20%), but without statistical differences (P>0.05). The 3-year survival rate of Duke’s C and D patients in proglumide group (73.3%) was obviously higher than that in control group (42.8%) (P<0.05). It indicated that administration of proglumide as adjuv ant therapy for patients with colorectal carcinomas, particularly the middle and late stage ones, could decrease the relapse or metastasis rate and prolong the survival period. But being a kind of weak gastrin receptor antagonist, only when the gastrin receptor level was high, could proglumide have an inhibitory effect. Therefore, its curable effect on large intestinal carcinoma awaits further evaluation more clinical observations.

Somatostatin is a kind of annular peptide hormone secreting by D cell. The main effect of somatostatin is to inhibit the growth, secretion and absorption of the mucosa of the gastrointestinal tract and to inhibit the release of gastrin, secretin, glucagon and growth hormone. Itzeu et al\(^{[26]}\) found with immunohistochemistry that there existed D cell in the mucosa of colorectal carcinoma, and suggested that D cell in mucosa of large intestine might have local regulatory effect on the secretion of other hormones. Dy et al\(^{[27]}\) reported that SMS 20.995 could significantly and concentration-dependently inhibit the growth of transplanted human colonic carcinoma from LIM2405 and LIM2412 cell line. We found that the volume, weight, DNA and protein content in carcinoma cell, cell amount and proliferation index of S and G2M phase in SMS group and SMS+PG group were markedly lower than those in PG group and control group, and markedly higher in PG group than those in control group. The cell amount of G1/G0 phase in SMS group and SMS+PG group was significantly higher than that in PG group and control group. This demonstrated that octapeptide somatostatin had a negative regulatory effect on the transplanted carcinoma, and it could not only inhibit the growth of transplanted human colonic carcinoma from SW480 cell line directly but also inhibit the growth-promoting effect of gastrin on the transplanted carcinoma. The half-life period of somatostatin is shorter than two minutes, so it can not be used pharmaceutically, but its analog manually synthesized such as SMS 201-995 and RC-160 have been used in clinical practice. Having treated 55 pati ents with advanced digestive tract carcinomas who could not endure chemotherapy with SMS201-995, Casciu et al\(^{[28]}\)found that SMS201-995 could relieve symptom and improve the quality of life and prolong the survival.

Colorectal carcinoma is a common kind of malignant carcinoma. The carcinoma was mostly in middle or advanced stage when patients first came to see a doctor. Treatment of gastrin-sensitive patients with colonic carcinoma with gastrin receptor or antagonists such as proglumide and somatostatin as adjuvant therapy is expected to prolong the survival of the patients and to raise the curative effect and to create new approaches for non-cytotoxic therapy such as hormonotherapy of patients with colorectal carcinoma.

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