JBUON 2019; 24(4): 1595-1600
ISSN: 1107-0625, online ISSN: 2241-6293 • www.jbuon.com
Email: editorial_office@jbuon.com

ORIGINAL ARTICLE

Correlations of gastrointestinal hormones with inflammation and intestinal flora in patients with gastric cancer

Gang Zhou, Jing Yang

Department of Medical Oncology in South Building, Chinese PLA General Hospital, Beijing, China.

Summary

Purpose: *To investigate the correlations of gastrointestinal hormones with inflammation and intestinal flora in patients with gastric cancer.*

Methods: *The data of patients with gastric cancer in the Department of Oncology and people with normal physical examination in our hospital were included. All patients received FOLFOX4 chemotherapy. The expression levels of gastrointestinal hormones and inflammatory cytokines were compared between the two groups, and the changes in the intestinal flora spectrum were analyzed. Two-sample t-test was used for the comparison between groups. Pearson's test was used for correlation analysis. P<0.05 showed statistical significance.*

Results: *The levels of serum gastrin-17 (G17) and pepsinogen II (PG II) detected in gastric cancer patients were higher than those in the control group, and the higher the tumor stage, the higher the expression levels. After therapy with PG I, the G17 and PG II levels increased. Moreover, the levels of serum interleukin-6 (IL-6) and IL-17 in patients with gastric cancer were higher than those in normal controls, and the higher the tumor stage, the higher the expression levels. After therapy with IL-6, the IL-6 and IL-7 levels were reduced. In addition, in gastric cancer patients, the numbers of Bifidobacteria, Lactobacilli and bacilli or cocci were apparently decreased, and were markedly increased after therapy, while those of Escherichia coli, Staphylococci, Enterococci and Peptostreptococci were significantly increased, and were evidently decreased after therapy. The results revealed that G17 had positive correlations with IL-6 and IL-17, PG II was positively correlated with IL-17, and G17 was negatively related to the numbers of Bifidobacteria and Lactobacilli.*

Conclusions: *Gastrointestinal hormones are involved in the occurrence and development of gastric cancer, and they have certain correlations with the inflammation and intestinal flora leading to the tumor genesis.*

Key words: *gastric cancer, gastrointestinal hormone, serum inflammatory cytokine, intestinal flora*

Introduction

As one of the major causes of cancer-related death, gastric cancer develops through a process involving multiple factors and steps [1]. Gastric cancer is primarily caused by Helicobacter pylori infection, which leads to mucosal inflammation and atrophy, thus ultimately causing cancer. It has been found that the gastric acid secretion will be reduced by the excessive growth of bacteria [2]. These bacteria may promote the production of nitrites and result in the accumulation of carcinogenic N-Nitrosocompound [3]. Hence, it is believed that the growth of bacteria triggers gastric cancer. The human intestinal flora consists of numerous bacterial species. Under physiologic conditions, the microbiota is crucial for human health, which participates in energy metabolism, nutrient absorption, maturation of the intestinal immune system and pathogen protection. Changes in the microbiota may be associated with cancer. A large amount of bacteria exists in the human stomach

Corresponding author: Jing Yang, MD. Department of Medical Oncology in South Building, Chinese PLA General Hospital. 28 Fuxing Rd, Haidian District, 100853 Beijing, China.
Tel: + 86 013911686070, Email: yangjing_ng_301@126.com
Received: 20/09/2018; Accepted: 30/11/2018

in addition to Helicobacter pylori, which are dominated by protease-producing bacteria, Firmicutes, Bacteroides, Fusobacteria and Actinomycetes. Despite quite significant variation in the most abundant bacteria among individuals, the gastric microbiota may have a correlation with the development of gastric cancer. Compared with that in specific pathogen-free mice, gastric cancer induced by Helicobacter pylori occurs later in sterilized transgenic mice. Interventions with antibiotic treatment can delay the occurrence of gastric cancer in transgenic mice without the influence on the infection of Helicobacter pylori. Feeding sterile transgenic mice with artificial intestinal flora accelerates cancer. Nevertheless, the study on the microbiota in gastric cancer is not deep enough, and the diversity, structure and composition of microorganisms in gastric cancer remain unclear.

Numerous studies have shown that hypergastrinemia (caused by helicobacter pylori infection) is positively related to human and mouse gastric cancer [4]. Hypergastrinemia and Helicobacter pylori infection synergistically promote the occurrence of gastric cancer in transgenic mice (INS-GAS) with overexpression of gastrin [5]. The roles of gastrointestinal hormones in the occurrence and development of gastric cancer has always been a controversial topic in the scientific community.

Gastric cancer normally occurs in the pylorus, but it also occurs in the corpus and fundus of stomach in 25% of the cases. Most clinical symptoms of gastric cancer are non-specific, so most patients are diagnosed in advanced stage. Generally, cancer cells are one of the sources of inflammatory cytokines and growth factors [6]. A sustained inflammatory process leads to proliferation of tumor cells, formation of blood vessels and inhibition of apoptosis. Vascular endothelial growth factors accelerate the formation of tumor blood vessels, and matrix metalloproteinases promote the tumor's expansion and spread to adjacent tissues, so as to stimulate the metastasis [7]. The occurrence of gastric cancer also has a close correlation with inflammation, but there is no research dealing with the effects of gastrointestinal hormones, inflammation and intestinal flora in the occurrence and development of gastric cancer.

Methods

Data of clinical cases

A total of 128 patients with gastric cancer hospitalized in the Department of Oncology of our hospital were included. This study was approved by the ethics committee of Chinese PLA General Hospital. Signed informed consents were obtained from all participants before the study entry. Inclusion criteria for subjects: (1) patients who were definitely diagnosed with gastric cancer by pathology, (2) patients whose clinical and pathological data were true and complete, and those whose histopathological specimens were available, and (3) patients with no family genetic history and no fatal diseases of the heart, lung, liver, kidney and other organs. Exclusion criteria: (1) pregnant and lactating women, (2) patients with severe cardiovascular and cerebrovascular diseases, or (3) patients with severe liver or kidney dysfunction or thyroid dysfunction.

Besides, the data of 50 people with normal physical examination results in the same period formed the control group. The basic data of the experimental and the control group are shown in Table 1.

All patients received FOLFOX4 combination chemotherapy (oxaliplatin/5-fluorouracil/leucovorin) [8].

Determination of intestinal flora

Indicator determination methods: (1) Preservation of specimens: 3-5 g fresh feces were collected from healthy people and patients before treatment under aseptic condition, which were sent to be inspected within 0.5 h. (2) Detection method for intestinal flora: 3-5 g fresh feces were collected by means of the aseptic method, and bacterial culture was carried out in the selective medium plate (Bifidobacterium: BBL agar medium, Lactobacillus: MRS agar medium, Enterococcus: m-Enterococcus agar medium, Escherichia coli: LB agar medium, Staphylococcus: CHAPMAN agar medium, yeast: YPD agar medium, Bacteroides: Nissui medium, and Peptostreptococcus: PS agar medium) to identify the intestinal flora. In light of different growth habits of aerobic and anaerobic bacteria, the culture was conducted strictly according to the suitable growth environment of these bacteria. Finally, the amount of bacteria in the fecal dilution after culture was calculated. (3) Detection of the proportions of fecal bacilli and cocci via Gram staining method: Feces were processed by Gram staining method (including initial staining, mordant dyeing, decolorization and counterstaining). Results: Under oil immersion lens, the Gram-positive bacteria were purple, and the Gram-negative bacteria were red. Some parts of the representative field

Table 1. Basic data of the experimental group and the control group

		Gender	
		Male (n)	*Female (n)*
Experimental group	Stage I-II (group B)	32	30
	Stage III-IV (group C)	30	26
Control group	Group A	22	28

Table 2. Levels of gastrointestinal hormones in the experimental group and the control group

Detection indicator	*G17 (pmol/L)*	*PG I (μg/L)*	*PG II (μg /L)*
Group A (n=50)	10. 31±2.07	98. 42±7.91	13. 17±1.87
Group B (n=62)	21. 16±3.65	79. 83±4.51	15. 94±3.24
Group C (n=66)	31. 17±2.63	68. 23±4.33	17. 21±2.25
t	21.995	18.035	6.302
p	<0.05	<0.05	<0.05

G17: gastrin, PGI/II: pepsinogen I/II

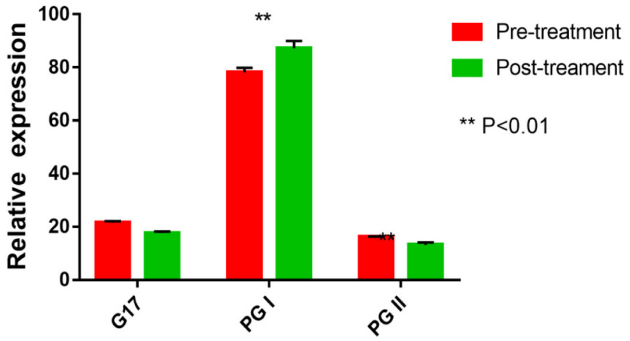

Figure 1. Changes in gastrointestinal hormones before and after FOLFOX4 chemotherapy.

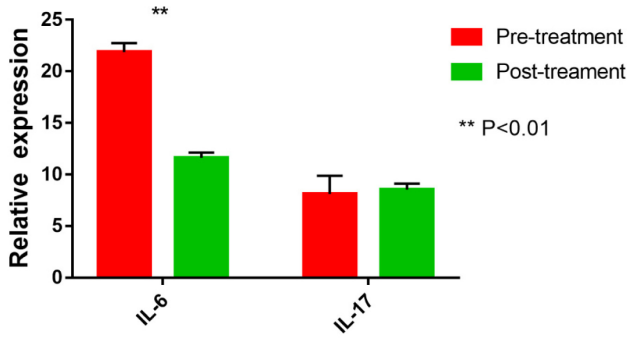

Figure 2. Changes in inflammatory cytokines before and after FOLFOX4 chemotherapy.

Table 3. Levels of inflammatory cytokines in each group

Detection indicator	*IL-6*	*IL-17*
Group A (n=50)	3.42±0.78	3.87±0.83
Group B (n=62)	10.76±2 41	7.94±1.39
Group C (n=66)	28.97±3.58	12.51±5.67
t	49.035	24.392
p	<0.05	<0.05

of view were selected for bacterial classification and counting, and 100-200 bacteria were needed to calculate the proportions of bacilli and cocci.

Determination of gastrointestinal hormones and inflammatory cytokines

Collection of serum specimens

In the morning, 5 mL fasting peripheral venous blood were taken from the subjects. The serum samples were separated by centrifugation at a radius of 15 cm, a speed of 3000 r/min and a time of 10 min, and they were placed at -80°C for storage and detection.

Detection methods

The levels of gastrin-17 (G17) and pepsinogen (PG) were determined by means of double antibody sandwich enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, MN, USA), and interleukin-6 (IL-6) and IL-17 levels were determined using ELISA. All procedures for detection were performed in strict accordance with the kit instructions.

Statistics

SPSS 22.0 software package (IBM, Armonk, NY, USA) was used for statistical processing of experimental data. Measurement data were expressed as mean ± standard deviation (x±s), and two-sample *t*-test was used for comparison between groups. Pearson method was applied for the correlation analysis, and a two-sided 95% confidence interval was applied in all tests. P<0.05 showed that the difference was statistically significant.

Results

Changes in gastrointestinal hormones

Levels of gastrointestinal hormones in different groups

The levels of serum G17 and PG II detected in the experimental group were higher than those in the control group on average, and in the experimental group, these levels detected in patients at stage III-IV were higher than those in patients at stage I-II, displaying statistical significance (p<0.05). However, the level of serum PG I detected in the experimental group was lower than that in the control group, and in the experimental group, this level detected in patients at stage III-IV was lower than that in patients at stage I-II, and the difference was statistically significant (p<0.05) (Table 2).

Table 4. Comparison of the number of the intestinal flora between the experimental group and the control group (cfu/mL)

Bifidobacterium	*Lactobacillus*	*Enterococcus*	*Escherichia coli*	*Staphylococcus*	*Peptostreptococcus*	*Bacillus/coccus*
9.87 ± 0.69	9.35 ± 0.61	8.28 ± 0.41	7.39± 0.41	3.87± 0.32	8.13 ± 0.65	4.24 ± 0.11
7.99± 0.80	7.81 ± 0.52	9.25 ± 0.58	8.30± 0.70	4.73± 0.57	8.80± 0.55	3.10± 0.19
5.99± 0.70	6.82 ± 0.62	11.25± 0.51	9.20± 0.72	5.73± 0.53	9.72± 0.63	2.10± 0.15
9.54	11.32	2.98	4.33	6.01	4.26	4.65
<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01

Table 5. Comparison of the number of the intestinal flora before and after FOLFOX4 chemotherapy (cfu/mL)

Bifidobacterium	*Lactobacillus*	*Enterococcus*	*Escherichia coli*	*Staphylococcus*	*Peptostreptococcus*	*Bacillus/coccus*
7.87 ± 0.69	7.35 ± 0.61	9.88 ± 0.41	8.39± 0.41	6.87± 0.32	8.83 ± 0.65	3.24 ± 0.11
8.99± 0.80	7.81 ± 0.52	9.95 ± 0.58	7.66± 0.70	5.73± 0.57	8.80± 0.55	3.10± 0.19
9.64	13.32	0.98	4.33	6.04	1.26	0.65
<0.01	<0.01	>0.05	<0.01	<0.01	>0.05	>0.05

Table 6. Correlation of gastrointestinal hormones with inflammatory cytokines

Group	*r*	*p*
G17 vs. IL-6	0.834	0.001
G17 vs. IL-17	0.789	0.032
PG I vs. IL-6	-0.112	0.067
PG I vs. IL-17	-0.443	0.087
PG II vs. IL-6	0.673	0.001
PG II vs. IL-17	0.234	0.176

Table 7. Correlation of gastrointestinal hormones with intestinal flora

Group	*r*	*p*
G17 vs. Bifidobacterium	-0.834	0.001
G17 vs. Lactobacillus	-0.789	0.032
G17 vs. Escherichia coli	0.432	0.131
G17 vs. Staphylococcus	0.421	0.231
PG I vs. Bifidobacterium	0.112	0.067
PG I vs. Lactobacillus	0.443	0.087
PG I vs. Escherichia coli	-0.211	0.211
PG I vs. Staphylococcus	-0.542	0.101
PG II vs. Bifidobacterium	-0.673	0.061
PG II vs. Lactobacillus	-0.234	0.176
PG II vs. Escherichia coli	0.231	0.191
PG II vs. Staphylococcus	0.441	0.061

Changes in gastrointestinal hormones before and after FOLFOX4 chemotherapy

The changes in gastrointestinal hormones in patients with gastric cancer before and after FOLFOX4 chemotherapy were compared. The results revealed that there were no evident differences in the levels of G17 and PG II detected before and after FOLFOX4 chemotherapy (Figure 1).

Inflammatory cytokines

Levels of inflammatory cytokines in different groups

The levels of serum IL-6 and IL-17 detected in the experimental group were higher than those in the control group on average, and in the experimental group, these levels in patients at stage III-IV were higher than those at stage I-II, showing statistical differences (p<0.05) (Table 3).

Changes in inflammatory cytokines before FOLFOX4 chemotherapy

The changes in inflammatory cytokines in patients with gastric cancer before and after FOLFOX4 chemotherapy were compared, which revealed that the level of IL-6 detected was reduced after chemotherapy, while no statistical difference in the level of IL-17 detected was found before and after FOLFOX4 chemotherapy (Figure 2).

Intestinal flora

Intestinal flora spectra in different groups

The differences in intestinal flora spectra among groups were compared. Comparison of the number of intestinal flora spectra between the experimental and the control group demonstrated that Bifidobacteria, Lactobacilli and bacilli or cocci were markedly reduced (p<0.01), while Escherichia coli, Staphylococci, Enterococci and Peptostreptococci were remarkably increased (p<0.01) (Table 4).

Changes in the intestinal flora before and after FOLFOX4 chemotherapy

The differences in intestinal flora spectra in

the experimental group before and after FOLFOX4 chemotherapy were compared. Comparison of the number of intestinal flora spectra before and after FOLFOX4 chemotherapy verified that Bifidobacteria, Lactobacilli and bacilli or cocci were evidently increased ($p<0.01$), while Escherichia coli, Staphylococci, Enterococci and Peptostreptococci obviously declined ($p<0.01$), displaying statistically significant differences (Table 5).

Correlations of gastrointestinal hormones with inflammatory cytokines and intestinal flora

Correlations of gastrointestinal hormones with inflammatory cytokines and intestinal flora in gastric cancer patients were analyzed. What was found was that G17 has positive correlations with IL-6 and IL-17 ($p<0.05$), and PG II was positively correlated with IL-17 ($p<0.001$), but PG I was not significantly associated with the expression level of inflammatory cytokines ($p>0.05$) (Table 6). G17 was negatively related to the numbers of Bifidobacteria and Lactobacilli ($p<0.05$), but not obviously correlated with the numbers of Escherichia coli and Staphylococci ($p>0.05$). No significant correlations of PG I and PG II with the intestinal flora were found ($p>0.05$) (Table 7).

Discussion

Gastric cancer is mainly caused by the helicobacter pylori infection, which exerts its primary effect on inducing oxidative mucosal atrophy. Atrophic gastritis leads to a decrease in gastric acid secretion, hypoacidity and secondary hypergastrinemia. This study indicated that the level of G17 in patients with gastric cancer was higher than that in normal controls, and the higher the tumor stage, the higher the expression level, suggesting that hypergastrinemia is more likely to occur in patients with gastric cancer. It has been identified in previous studies that neuroendocrine (NE) markers, more specifically enterochromaffin-like (ECL) cell markers, are expressed in gastric cancer patients, especially in those with diffuse gastric cancer [8], whose pathogenesis is associated with gastrin. There are a few studies reporting hypergastrinemia in gastric cancer patients [9]. It is highly possible that diffuse gastric cancer is derived from differentiated ECL cells, and the intestinal type is related to atrophic oxidative gastritis [10], but this type of gastric cancer rarely expresses NE markers, so it is more likely to develop from stem cells. Nevertheless, gastrin may be a crucial stimulator of intestinal-type gastric cancer in the carcinogenic process, and it plays a role in cells through the gastrin receptor in cells or indirectly mediating ECL cells.

This study also revealed the enrichment of six bacterial genera in gastric cancer, which is consistent with the results in recent studies [11], indirectly reflecting a decrease in bactericidal capacity due to reduced acid production in the stomach. Some species of lactic acid bacteria are used as probiotics to play a role in preventing pathogen infection, alleviating inflammation and regulating microbiota [12,13]. Furthermore, lactic acid bacteria can also induce inflammatory damage to epithelial cells and illuminate that the changes in the intestinal flora are related to the induction of tumor inflammation to some extent. According to previous studies, Escherichia coli produce a genotoxic toxin promoting the development of colon cancer in mice [14], which, therefore, may participate in the development of gastric cancer.

Generally, inflammation is related to cancer development, and gastric cancer seems to be particularly sensitive to inflammation [15,16]. Inflammation can stimulate the development of tumor lesions, thus leading to the formation of malignant phenotypes. In gastrointestinal tumors, the activation level of signal transducer and activator of transcription 3 (STAT3) is important for increase in the tumor size and proliferation in mouse models, and IL-6 is the most important activator of STAT3 cascade [17]. Sufficient evidence has confirmed that inflammation has a correlation with the development of gastric cancer and is an important risk factor for gastric cancer, but its exact biological mechanism remains not clear [18-20]. Many studies have examined the correlations of IL-6 with gastric cancer and other cancer types, so as to identify that IL-6 exerts effects in the development and maintenance of tumor cells. Gastric cancer cells secrete IL-6, and the increase of IL-6 in serum and gastric cancer tissues regulates the tumor growth and development in the autocrine cycle.

It is of important clinical significance to understand the basic role of gastrointestinal hormones in the development of gastric cancer. Therefore, long-term treatment for patients, especially for children and young people, should be avoided due to their long life expectancy, and inhibiting gastric secretion will lead to hypergastrinemia. In the future, gastrointestinal hormone antagonists are likely to be applied to treat hypoxic atrophic gastritis and hypergastrinemia in young patients for cancer prevention. Moreover, the stage of tumor development where gastrin loses efficacy must be elucidated, so as to determine whether a gastrin antagonist is available for treatment.

Conclusions

In summary, this study demonstrates that gastrointestinal hormones participate in the occurrence and development of gastric cancer, and they have certain correlations with the inflammation and intestinal flora leading to tumor development.

Conflict of interests

The authors declare no conflict of interests.

References

1. Correa P. Human gastric carcinogenesis: a multistep and multifactorial process--First American Cancer Society Award Lecture on Cancer Epidemiology and Prevention. Cancer Res 1992;52:6735-40.
2. Sanduleanu S, Jonkers D, De Bruine A, Hameeteman W, Stockbrugger RW. Non-Helicobacter pylori bacterial flora during acid-suppressive therapy: differential findings in gastric juice and gastric mucosa. Aliment Pharmacol Ther 2001;15:379-88.
3. Koliarakis I, Psaroudaki A, Nikolouzakis TK et al. Intestinat microbiota and colorectal cancer: A new aspect of research. JBUON 2018;23:1216-34.
4. Takaishi S, Tu S, Dubeykovskaya ZA et al. Gastrin is an essential cofactor for helicobacter-associated gastric corpus carcinogenesis in C57BL/6 mice. Am J Pathol 2009;175:365-75.
5. Takaishi S, Cui G, Frederick DM et al. Synergistic inhibitory effects of gastrin and histamine receptor antagonists on Helicobacter-induced gastric cancer. Gastroenterology 2005;128:1965-83.
6. Hussain SP, Harris CC. Inflammation and cancer: an ancient link with novel potentials. Int J Cancer 2007;121:2373-80.
7. Ikeda M, Furukawa H, Imamura H et al. Surgery for gastric cancer increases plasma levels of vascular endothelial growth factor and von Willebrand factor. Gastric Cancer 2002;5:137-41.
8. Garrido M, Melgoza G, Calindo H et al. Treatment of advanced gastric cancer with oxaliplatin plus 5-fluorouracil/leucovorin (FOLFOX4 chemotherapy). Rev Med Chil 2007;135:1380-7.
9. Waldum HL, Aase S, Kvetnoi I et al. Neuroendocrine differentiation in human gastric carcinoma. Cancer 1998;83:435-44.
10. Rakic S, Milicevic MN. Serum gastrin levels in patients with intestinal and diffuse type of gastric cancer. Br J Cancer 1991;64:1189.
11. El-Zimaity HM, Ota H, Graham DY, Akamatsu T, Katsuyama T. Patterns of gastric atrophy in intestinal type gastric carcinoma. Cancer 2002;94:1428-36.
12. Eun CS, Kim BK, Han DS et al. Differences in gastric mucosal microbiota profiling in patients with chronic gastritis, intestinal metaplasia, and gastric cancer using pyrosequencing methods. Helicobacter 2014;19:407-16.
13. Citar M, Hacin B, Tompa G et al. Human intestinal mucosa-associated Lactobacillus and Bifidobacterium strains with probiotic properties modulate IL-10, IL-6 and IL-12 gene expression in THP-1 cells. Benef Microbes 2015;6:325-36.
14. Zhang H, Wang H, Shepherd M et al. Probiotics and virulent human rotavirus modulate the transplanted human gut microbiota in gnotobiotic pigs. Gut Pathol 2014;6:39.
15. Arthur JC, Perez-Chanona E, Muhlbauer M et al. Intestinal inflammation targets cancer-inducing activity of the microbiota. Science 2012;338:120-3.
16. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011;144:646-74.
17. Fichtner-Feigl S, Kesselring R, Strober W. Chronic inflammation and the development of malignancy in the GI tract. Trends Immunol 2015;36:451-9.
18. Grivennikov S, Karin E, Terzic J et al. IL-6 and Stat3 are required for survival of intestinal epithelial cells and development of colitis-associated cancer. Cancer Cell 2009;15:103-113.
19. Sanchez-Zauco N, Torres J, Gomez A et al. Correction to: Circulating blood levels of IL-6, IFN-gamma, and IL-10 as potential diagnostic biomarkers in gastric cancer: a controlled study. BMC Cancer 2017;17:669.
20. Plummer M, de Martel C, Vignat J, Ferlay J, Bray F, Franceschi S. Global burden of cancers attributable to infections in 2012: a synthetic analysis. Lancet Glob Health 2016;4:e609-16.
21. Chen G, Tang N, Wang C et al. TNF-alpha-inducing protein of Helicobacter pylori induces epithelial-mesenchymal transition (EMT) in gastric cancer cells through activation of IL-6/STAT3 signaling pathway. Biochem Biophys Res Commun 2017;484:311-7.