Expression of 5-HT Relates to Stem Cell Marker LGR5 in Patients with Gastritis and Gastric Cancer

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Abstract
Background 5-Hydroxytryptamine (5-HT) and stem cells marker G-protein-coupled receptor 5 (LGR5) are associated with gastrointestinal inflammation and tumorigenesis. But the relationship between 5-HT and LGR5 is unclear.

Objective To explore the expression and correlation of 5-HT and LGR5 in gastric mucosa of patients with gastritis and gastric cancer (GC).

Methods A total of 41 patients with GC and 98 patients with chronic gastritis were included in this study. The expression of TPH1 mRNA, LGR5 mRNA and β-catenin mRNA in gastric mucosa were explored by Real-time Quantitative polymerase chain reaction (qPCR). 5-HT-positive cells and LGR5-positive cells in gastric mucosa were detected by immunohistochemistry stains. The co-localization of 5-HT and chromogranin A (CgA), 5-HT receptor4 (5-HTR4) and LGR5 were detected by multiplex immunofluorescence.

Results The expression of 5-HT and LGR5 in patients with GC was significantly higher than patients with chronic gastritis (p < 0.05). The positive rate of 5-HT and LGR5 increased sequentially in the patients with non-atrophic gastritis, intestinal metaplasia and GC, which were 18.52%, 35.56% and 75.61% for 5-HT, and 27.78%, 40.91% and 95.12% for LGR5, respectively. The expression of 5-HT and LGR5 was positively correlated in gastritis and GC patients (p < 0.05). Moreover, the expression level of TPH1 mRNA and LGR5 mRNA was also positively correlated in gastritis patients (r = 0.7377, p < 0.001). Besides, 5-HT was partially co-localized with CgA, and 5-HTR4 was co-localized with LGR5 in gastric mucosa.

Conclusion The increase of 5-HT synthesis in gastric mucosa may have an impact on LGR5-positive gastric epithelial stem cells.

Keywords Gastric cancer · Gastritis · 5-HT · LGR5 · Stem cells

Introduction
5-Hydroxytryptamine (5-HT), also known as serotonin, is an indole derivative which is first found in serum In vivo, 5-HT (95%) mainly derives from enterochromaffin cells (ECs), with relatively few from enteric neurons. Tryptophan hydroxylase1 (TPH1) is a rate-limiting enzyme for 5-HT synthesis in ECs. In addition to immunomodulatory effects, 5-HT can accelerate the progress of mitosis through
combining different receptors in bladder cancer, prostate cancer, breast cancer and other cancer tissues, and be involved in the occurrence, development and metastasis of tumors [1–3]. Gastric cancer (GC), especially gastric adenocarcinoma, is one of the most common malignant tumors in human beings. "Chronic superficial gastritis-chronic atrophic gastritis-intestinal metaplasia, atypical hyperplasia-GC", referred to as "Correa cascade", is accepted as an evolution of intestinal non-cardiac GC [4]. However, the biological function of 5-HT in gastric adenocarcinoma is poorly understood.

LGR5, also called GPR49, is a G protein-coupled receptor and target gene of the Wnt signal pathway. It has enormous leucine repeat sequences. LGR5-positive cells are present in a variety of tissues such as hair follicles, mammary glands, kidneys, colon, and pylorus of the stomach. The LGR5-positive cells have the nature of self-renewal and play an important role in the maintenance of epithelial homeostasis and injury repair [5, 6]. LGR5 is considered as a marker of intestinal stem cells. In the colonic crypts, Paneth cells control the number of LGR5-positive stem cells by modulating the expression of signaling molecules such as Epidermal Growth Factor, Transforming growth factor, Wnt3 and Notch-ligand Dll4 [7]. Interestingly, LGR5-positive cells are located next to the 5-HT-positive ECs. In normal gastric mucosa, LGR5-positive cells are predominantly located at the base of the gastric lacunae, which is similar to the histological localization of 5-HT-positive ECs. We hypothesized that 5-HT-positive ECs may affect the function of LGR5-positive epithelial stem cells by secreting 5-HT in the antral mucosa.

In this study, we assessed the relationship between 5-HT and LGR5. The expression levels of 5-HT and LGR5 were examined in gastric mucosa of patients with chronic gastritis and GC. Next, co-localization of 5-HT with CgA and LGR5 were detected separately.

**Materials and Methods**

**Research Objects and Tissue Samples**

We used a case-control design involving the comparison of 41 patients with GC and 98 patients with chronic gastritis in the study. All cases were collected from the department of Gastroscope and the department of Pathology from 2018 to 2019, at the Peking University Aerospace School of Clinical Medicine. All patients did not take gastric motility drug or antidepressants within 3 months prior to gastroscopy. They had no history of gastrointestinal surgery or gastrointestinal tumors and were not first-degree relatives of patients with malignant tumors.

Two pieces of the mucosa were taken at gastric antrum under gastroscope and washed immediately with sterile PBS. One piece of tissue was frozen quickly in liquid nitrogen and stored at -80 °C awaiting use. The other piece of tissue was immersed in formalin solution to prepare a paraffin section. The study was approved by the Ethics Committee of the Peking University Aerospace School of Clinical Medicine, and all participants signed the informed consent.

**RNA Extraction and qPCR**

The total RNA was extracted using the RNAprep Pure Tissue Kit (Cat. DP431, TIANGEN, China), and complementary DNA was synthesized using the FastQuant RT Kit (With gDNase) (Cat. KR106, TIANGEN, China). Quantitative PCR was performed using QuantiNova SYBR Green PCR Kit (QIAGEN, Germany). The primer sequences were as follows: The GAPDH 5′-CCACATCGCTCAGACATCAT-3′ (forward) and 5′-GGCAACATATCCACTTTACCCAGA GT-3′ (reverse); Tph1 5′-TGCAAAGGAAAAGATGAG AGAATTTA-3′ (forward) and 5′-CTGGTTATGCTCTTG GTGTCCTTC-3′ (reverse); Lgr5 5′-TGGTGGACACAGGG AGACCTGGAGAT-3′ (forward) and 5′-GAAAAGCGAAC AGGCAGTTAGATG-3′ (reverse); and β-catenin 5′-GGA CTTTGAGGAAGATGG-3′ (forward) and 5′-ATCTGTC TGGAAGGTTGGACACG-3′ (reverse). PCR reaction condition (20 µl, 40 cycles) was 95 °C, 2 min; 95 °C, 5 s; 60 °C, 10 s. The products were sent to Meggie Biology for sequencing and the data were analyzed by Bio-Rad CFX Manager 3.1 software. The relative expression of the target genes was analyzed with 2−ΔCt.

**Immunohistochemistry and Multiple Immunofluorescence of Stomach Tissues**

Sections of formalin-fixed, paraffin-embedded human gastric antral tissue were fixed for 20 min in an incubator at 60 °C, dehydrated for 10 min, and then immersed continuously in 100% ethanol, 95% ethanol and 70% ethanol for 20 s. Antigen repair was performed using sodium citrate buffer (pH 6.0) for LGR5 staining and Tris–EDTA buffer for 5-HT staining. Then, sections were blocked with 1% sheep serum for 30 min to eliminate non-specific antibodies. Blocking serum was discarded and tissue sections were incubated with monoclonal anti-human 5-HT antibody (1:100, Abcam, UK), monoclonal anti-human LGR5 antibody (1:100, Abcam, UK) overnight at 4 °C. The sections were incubated with HRP-labeled secondary antibody (Solarbio, China),at room temperature for 1 h and then treated with DAB for color development.. Image analysis was performed using Image J software (Rawak Software Inc., NIH, USA).

For multiple immunofluorescence, sections were incubated separately overnight at 4°C with either monoclonal
anti-human 5-HT antibody (1:200, Abcam, UK) and polyclonal anti-human CgA antibody (1:200, Abcam, UK) or polyclonal anti-human 5-HTR4 antibody (1:200, Abcam, UK) and monoclonal anti-human LGR5 antibody (1:200, Abcam, UK). The slides were rinsed in PBS, incubated with secondary antibody for 30 min at 37 °C, and stained with 4′,6-diamidino-2-phenylindole (DAPI) for 2 min. The slides were encapsulated with anti-fluorescence decay and observed under an inverted fluorescence microscope (Leica, Germany).

**Scoring of Immunohistochemistry and Immunofluorescence Stains**

Two fields with the highest positive intensity were randomly selected after observing the whole tissue sections (×200). The number of positive cells in each field was counted. Staining intensity was classified into four grades: Non-pigmented (0); pale yellow (1); tan (2); and brown (3). Grades 1 to 3 were considered as positive expression. The count of positive cells was divided into five types: non-positive cells (0); scattered positive cells < 1% (1); positive cells 2% -10% (2); positive cells 11% -50% (3); positive cells 51% -80% (4); and positive cells > 80% (5). The evaluation was analyzed by multiplying the percentage of positive cells and the intensity of cytoplasmic staining. The results were marked as: negative (0–2); weak positive (3–4); moderate positive (5–8); and strong positive (> 8).

At least ten positive cells were counted to determine the co-localization of 5-HT-positive cells with CgA-positive cells, and 5-HTR4-positive cells with Lgr5-positive cells in gastric mucosa.

**Statistical Analysis**

T-test or Pearson correlation analysis was performed for normally distributed data, Wilcoxon rank sum test or Spearman correlation analysis was performed for non-normally distributed data. SAS 9.2 software (SAS INSTITUTE INC, USA) was used to analyze the data. p value < 0.5 were considered as significant.

**Results**

**Basic Data**

In this study, a total of 139 patients were enrolled, including 98 (70.5%) patients with chronic gastritis and 41 (29.5%) patients with GC. The mean age of patients with chronic gastritis was 55.11 ± 14.48 years, and the male/female ratio was about 1: 1. The mean age of GC patients was 70.68 ± 13.15 years, and the male/female ratio was about 2: 1. There was a statistically significant difference in age between the two groups (z = 5.3983, p < 0.01).

**Expression of 5-HT in Gastric Antral Mucosa**

As shown in Fig. 1, the expression of 5-HT was detected in both epithelial cells and lamina propria cells. Among 98 patients with chronic gastritis, 5-HT positive cases were 26.53% (26/98), and mainly were moderate and low intensity of staining (Fig. 1a, b). 75.61% (31/41) patients with gastric adenocarcinoma presented strong 5-HT-positive expression (Fig. 1c, d). The positive rate of 5-HT in the patients with non-atrophic gastritis, intestinal metaplasia and GC increased sequentially, which were 18.52%, 35.56%, and 75.61%, respectively.

**Expression of Stem Cell Marker LGR5 in Gastric Antral Mucosa**

To further explore whether the expressions of 5-HT and LGR5 are correlated in different gastric lesions, we performed immunohistochemical detection of LGR5. As shown in Fig. 2, LGR5-positive cells expressed in both the glandular epithelium and lamina propria which was similar to 5-HT-positive cells. Among the 98 patients with chronic gastritis, 33.67% (33/98) patients had positive expression of LGR5 (Fig. 2a, b). Among the 41 patients with gastric adenocarcinoma, 95.12% (39/41) patients had positive expression of LGR5 (Fig. 2c, d). The expression rates of LGR5 in the patients with non-atrophic gastritis, intestinal metaplasia and GC increased sequentially, which were 27.78%, 40.91%, and 95.12%, respectively. According to the results of 5-HT staining and LGR5 staining, the proportion of double-positive patients were the highest among the patients with gastric adenocarcinoma. However, the proportion of double-negative patients were higher among the patients with chronic gastritis.

The correlation between 5-HT or LGR5 expression and clinicopathological parameters in patients with gastritis was shown in Table 1. Moreover, statistical analysis revealed a high correlation between the presence of 5-HT and LGR5 in both gastritis patients and GC patients as shown in the table below (Tables 2 and 3).

**Co-expression of 5-HT and CgA Protein, 5-HTR4 and LGR5 Protein in Gastric Mucosa**

To validate the correlation of 5-HT and LGR5, we examined the subcellular localization of the 5-HT and LGR5 in 1 patient with chronic gastritis and 2 patients with GC. CgA is a marker of enteroendocrine cells which secretes 5-HT. We first tested the co-localization between 5-HT and CgA in gastric mucosal cells. Multiple immunofluorescence images
showed both 5-HT and CgA proteins were diffusely cytoplasmic staining. The number of CgA-positive cells was significantly higher than the number of 5-HT-positive cells, which showed partial co-localization in gastric mucosa. Since 5-HTR4 acted as a functional receptor of 5-HT, next, the co-localization of LGR5 and 5-HTR4 was explored. As shown in Fig. 3, LGR5 protein was diffusely cytoplasmic staining, while the 5-HTR4 was granularly membrane staining. They were expressed as partially co-localized cells in gastric mucosa suggesting that there was 5-HT receptor on the membrane surface of LGR5-positive cells. We tested the slices of 3 different patients and counted the number of staining cells (Table 4).

**Correlation Between 5-HT and the Expression of LGR5 and β-Catenin**

We detected the expression of TPH1 mRNA, LGR5 mRNA and β-catenin mRNA in gastric antral mucosa from 52 patients with chronic gastritis by real-time PCR(Fig. 4a). The results showed that the expression level of TPH1 mRNA was positively correlated with the expression level of LGR5 mRNA ($r = 0.7377$, $p < 0.01$) (Fig. 4b), but not correlated with the expression level of β-catenin mRNA ($r = 0.2519$, $p = 0.0716$) (Fig. 4c).

**Discussion**

Previous studies have shown that both 5-HT and LGR5 played important roles in gastric mucosal inflammatory injury. Our study explored the relationship between 5-HT and LGR5 in the gastric carcinogenesis axis for the first time. We experimentally verified that the expression levels of 5-HT and Lgr5 were gradually increased in gastric mucosa of patients with Non-atrophic gastritis, atrophic gastritis and GC. Immunohistochemical staining of 5-HT and LGR5 protein showed that the positive rate of 5-HT in gastric adenocarcinoma patients was more than 2.9 times higher than in chronic gastritis patients (75.61% vs 26.53%), while the positive rate of LGR5 was almost 2.8 times more higher in gastric adenocarcinoma patients than in chronic gastritis patients (95.12% vs 33.67%). We also found the expression level of Tph1 mRNA was positively correlated...
with LGR5 mRNA in patients with chronic gastritis. Moreover, LGR5-positive cells had 5-HT receptor on the cell membrane, suggesting that LGR5-positive cells exerted a molecular structure of binding to 5-HT. 5-HT might take part in the biological function of gastric epithelial stem cells in patients with gastritis and GC.

Table 1  Correlation between 5-HT or Lgr5 expression and clinicopathological parameters in patients with gastritis

| Clinicopathological features | 5-HT                      | p value | Lgr5                      | p value |
|------------------------------|---------------------------|---------|---------------------------|---------|
|                              | Positive(%) | Negative(%) |       | Positive(%) | Negative(%) |       |
| Sex                          |             |           |       |             |           |       |
| Male                         | 14 (53.8)  | 30 (41.7) | 0.359| 14 (42.4)  | 30 (46.2) | 0.831|
| Female                       | 12 (46.2)  | 42 (58.3) |       | 19 (57.6)  | 35 (53.8) |       |
| Age                          |             |           |       |             |           |       |
| < 60                         | 17 (65.4)  | 39 (54.2) | 0.363| 11 (33.3)  | 31 (47.7) | 0.200|
| ≥ 60                         | 9 (34.6)   | 33 (45.8) |       | 22 (66.7)  | 34 (52.3) | 0.000|
| Helicobacter pylori          |             |           |       |             |           |       |
| Positive                     | 3 (11.5)   | 9 (12.5)  | 1.000| 4 (12.1)   | 8 (12.3)  | 1.000|
| Negative                     | 23 (88.5)  | 63 (87.5) |       | 29 (87.9)  | 57 (87.7) |       |
| Atrophy                      |             |           |       |             |           |       |
| Atrophic gastritis           | 15 (57.7)  | 27 (37.5) | 0.105| 16 (48.5)  | 26 (40.0) | 0.518|
| Non-atrophic gastritis       | 11 (42.3)  | 45 (62.5) |       | 17 (51.5)  | 39 (60.0) |       |

Fig. 2  Expression of Lgr5 in gastric mucosa by immunohistochemistry. a Lgr5-positive cells (red arrows) in the antral mucosa of patients with non-atrophic gastritis; b Lgr5-positive cells in gastric mucosa of patients with atrophic gastritis and intestinal metaplasia; c Lgr5-positive cells in the antral mucosa of patients with gastric cancer; d Lgr5-positive cells from lamina propria in gastric mucosa of patients with gastric adenocarcinoma; Original magnification ×200 (a–c)
5-HT is a key signaling molecule involved in the progress of gastrointestinal secretion, sensation, and peristalsis. It is produced by gastrointestinal ECs and usually stored in platelets by serotonin transporter (SERT). Once released by platelets, 5-HT can be involved in the development of peripheral inflammation through chemical induction and activation of immune cells [8]. Studies have shown that in inflammatory bowel disease (IBD), the transcriptional expression of TPH1 was elevated, leading to both increased 5-HT synthesis and decreased reuptake efficiency. The enhancement of 5-HT signaling results in persistent intestinal inflammation and associated manifestations [9]. Our former study showed that increased 5-HT synthesis was associated with mucosal inflammation in colorectal adenoma patients [10]. A previous study reported that a significant reduction in the severity of DSS-induced colitis in Tph1−/− mice [11].

It is well known that Helicobacter pylori (HP) is an independent risk factor for chronic gastritis and gastric cancer. In our study, there was no statistical difference between the

| Lgr5 | 5-HT Positive (%) | 5-HT Negative (%) | p Value |
|------|-------------------|-------------------|---------|
| Positive | 23 (88.5) | 10 (13.9) | 0.001 |
| Negative | 3 (11.5) | 62 (86.1) | |

Table 3 Correlation between 5-HT and Lgr5 expression in patients with gastric cancer

| Lgr5 | 5-HT Positive (%) | 5-HT Negative (%) | p Value |
|------|-------------------|-------------------|---------|
| Positive | 30 (100) | 9 (81.8) | 0.018 |
| Negative | 0 (0) | 2 (18.2) | |

Fig. 3 Co-localization of 5-HT and CgA protein, Lgr5 protein and 5-HT4R in gastric mucosa. a 5-HT-positive cells stain green (white arrow); b CgA-positive cells stain red (yellow arrow); c Merge images obtained from 5-HT-positive cells stain and CgA-positive cells stain (green arrows): 5-HT-positive cells colocalize partially with CgA-positive cells in the gastric mucosa (green arrows); d Lgr5-positive cells stain green (white arrow); E. 5-HTR4-positive cells stain red (yellow arrow); f Merge images obtained from Lgr5-positive cells stain and 5-HTR4-positive cells stain (green arrows): Lgr5-positive cells co-localized with 5-HTR4-positive cells partially. Original magnification ×200 (a–f)

Table 4 Multiple immunofluorescent results of 3 patients

| Sample | Cells | Case 1 | Case 2 | Case 3 |
|--------|-------|--------|--------|--------|
| Sample 1 | 5-HT-positive/CgA-positive cells | 43.2% (32/74) | 49.4% (40/81) | 6.25% (2/32) |
| Sample 1 | 5-HT-positive/CgA-negative cells | 4.1% (3/74) | 7.4% (6/81) | 21.9% (7/32) |
| Sample | 5-HT-negative/CgA-positive cells | 52.7% (39/74) | 43.2% (35/81) | 71.9% (23/32) |
| Sample 2 | 5-HTR4-positive/Lgr5-positive cells | 30.8% (20/65) | 33.8% (24/71) | 35.4% (28/79) |
| Sample 2 | 5-HTR4-positive/Lgr5-negative cells | 33.8% (22/65) | 38.0% (27/71) | 62.0% (49/79) |
| Sample 2 | 5-HTR4-negative/Lgr5-positive cells | 35.4% (23/65) | 28.2% (20/71) | 2.5% (2/79) |
expression of 5-HT and HP infection in gastritis patients. We noticed that there were few studies focusing on the function of 5-HT in HP-related gastritis. Some studies showed that the expression of serotonin containing endocrine cells (ECs) in the corporal gastric mucosa had no relationship with HP infection [12]. On the contrary, another study of Chinese medicine, showed that the expression of 5-HT and CgA in HP-infected gastritis patients with spleen-stomach damp-heat syndrome was higher than healthy control people [13].

5-HT and its receptors play an important role in the progression of malignant tumors. Jin X et al. showed that high expression level of 5-HT receptor 1D (HTR1D) was associated with poor prognosis in GC patients. Knockdown of HTR1D could disrupt the proliferation and migration of GC cells [14]. Lv et al. found that vortioxetine, a potent antagonist of 5-HT receptors and 5-HT transporters, could induce apoptosis and autophagy via the phosphoinositide 3-kinase/AKT pathway [15]. Similarly, studies have also shown that the application of 5-HT inhibitor fluoxetine could inhibit the proliferation, invasion and migration of gastric cancer cells [16, 17]. All these data suggested that 5-HT took part in the tumorigenesis and might be a new target.

LGR5 protein is a marker of stem cells in a variety of tissues. Tumor stem cells, or tumor initiating cells (TICs), are a group of cancer cells which have capabilities of self-renewal, asymmetric division and multidirectional differentiation [18]. It has been confirmed that TICs presented in multiple epithelial-derived solid tumor models such as colorectal cancer, breast cancer and lung cancer. Recurrent regeneration of gastric epithelium is driven by long-lived stem cells [19]. LGR5-positive cells were mainly located in the basal layer of mature pyloric glands. LGR5 was co-stained with keratin and CD44, indicating that LGR5-positive cells might be derived from epithelial lineage and stem cell lineage. In the lamina propria, LGR5 expressed sparsely and co-stained with vimentin and CD45, indicating LGR5-positive cells originated from leukocyte lineage [20]. It was postulated that LGR5-positive progeny cells from the basal portion of the gland migrated to the isthmus and proliferated rapidly [21]. Jennelle et al. found that an increased number of LGR5-positive stem cells were shown in patients with familial adenomatous polyposis (FAP) compared to healthy people [22]. There was a strong relationship between LGR5 and vascular invasion, lymph node metastases and poor overall survival. Studies confirmed that the presence of LGR5 was an unfavorable prognostic factor in colorectal cancer (CRC) [23]. In a study of Leushacke et al. ablation of LGR5-positive cells severely impacted epithelial stability. LGR5-positive master cells was defined as the primary source of GC cells [24]. The META analysis by Guo et al. also showed that LGR5 might be a potential prognostic factor for GC survival prediction [25]. Furthermore, the carcinogenesis of LGR5-positive cells was also demonstrated in animal models [21]. These findings were consistent with our study. The expression of LGR5 in GC patients was significantly higher than that in gastritis patients.

5-HT was synthesized and secreted by specialized enteroendocrine cells within the gastrointestinal mucosa. In our study, partially co-localization of 5-HT and CgA consistent with previous perceptions. Seven subtypes of 5-HT receptors (5-HTR), named 5-HTR1 to 5-HTR7, have been identified. Among these, 5-HTR3 and 5-HTR4 were vital receptors in gastrointestinal tract. Using immunofluorescent staining, we verified the expression of 5-HTR4 in gastric cells. More importantly, 5-HTR4 was particularly stained around the LGR5 protein on the membrane of double positive cells. Immunofluorescence staining verified that 5-HT might interact with LGR5-positive cells by recognizing and binding to 5-HT4R. These findings might provide new ideas for the mechanism of GC.

5-HT is thought to be an important factor for Wnt/β-catenin signaling pathway during axonal formation in Xenopus laevis. It was found that 5-HT antagonists (SB-258719) decreased the expression level of β-catenin in...
tumor cells, attenuated Wnt/β-catenin signaling pathway, and played an important role in tumorigenesis [26]. The study by Leushacke et al. have shown that 5-HT could initiate Wnt/β-catenin signaling. Blocking 5-HT signaling in mice not only inhibited self-renewal of colorectal cancer stem cells (CSCs), but also showed therapeutic effects on CRC [27]. Interestingly, LGR5 is a regulatory target gene for the Wnt/β-catenin signaling pathway. Whether 5-HT could interact with LGR5 by Wnt/β-catenin pathway is still controversial. Zhu et al. showed that LGR5-positive intestinal stem cells (ISCs) are regulated by 5-HT, which promoted Wnt/β-catenin signaling to self-renewal [28]. Previous research suggested that the expression of LGR5 was positively correlated with β-catenin in colorectal cancer [29]. In contrast, LGR5, in two other studies, played a negative regulatory role in Wnt/β-catenin signaling pathway in tumor tissue [30, 31]. In our study, the expression of Tph1 and LGR5 in gastric antral mucosa of patients with chronic gastritis was not statistically correlated with the expression of β-catenin gene. This result might be influenced by the sample size. Next, we will continue to expand the sample size and focus on signaling mechanisms.

In conclusion, our research suggests that increased 5-HT is associated with high expression of stem cell marker LGR5 in gastric mucosa of patients with chronic gastritis and GC for the first time. These data may provide a new idea for exploring the pathogenesis and targeted therapy of GC. However, our study has some limitations that future research can remedy. Firstly, due to constraints of ethical, we do not have a sample of healthy people as control group. Secondly, the regulatory mechanism of 5-HT and LGR5 has not been clarified. 5-HT inhibitor will be used to further explore the relationship between 5-HT and LGR5. In addition, in order to explore the therapeutic value, clinical explorations are still needed in the existing gastric and intestinal related research.

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