The Involvement of CXCL17-GPR35 in Gastric Cancer Initiation and Development

Yi-zhi Li

The First Affiliated Hospital of China Medical University

Ao-ran Liu

The First Affiliated Hospital of China Medical University

Song-yi Liu

The First Affiliated Hospital of China Medical University

Li-rong Yan

The First Affiliated Hospital of China Medical University

Yuan Yuan

the first affiliated hospital of china medical university

Qian Xu (✉ qxu@cmu.edu.cn)

First Hospital of China Medical University <https://orcid.org/0000-0001-8073-6656>

Primary research

Keywords: CXCL17, GPR35, CCL20, Gastric cancer, Prognosis

Posted Date: November 2nd, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-1029803/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Abstract

Background: C-X-C motif chemokine 17 (CXCL17), a newly identified chemokine, and its possible receptor, G-protein coupled receptor 35 (GPR35) were potential proteins involved in carcinogenesis; nevertheless, their involvement in gastric cancer (GC) initiation and development, which we aimed to evaluate in this study, remains undefined.

Methods and materials: CXCL17 and GPR35 expressions in normal gastric gland of non-atrophic gastritis (NAG-NOR), intestinal metaplasia of atrophic gastritis (AG-IM), IM adjacent to GC (GC-IM), and GC tissues was determined by immunohistochemistry. Their possible roles were analyzed by the Cancer Genome Atlas (TCGA) data mining and western blot.

Results: Either CXCL17 or GPR35 showed a strongest expression in GC-IM among the four types of tissues: CXCL17: GC-IM vs. AG-IM ($P < 0.001$), GC-IM vs. GC ($P < 0.001$); GPR35: GC-IM vs. AG-IM ($P < 0.001$), GC-IM vs. GC ($P < 0.01$). Their expressions were positively correlated in GC tissues (Spearman $r = 0.2933$, $P < 0.001$). CXCL17 expression was negatively correlated with the depth of tumor invasion ($P = 0.015$) and tumor maximum diameter ($P = 0.003$). Higher CXCL17 expression independently predicted for better survival in GC patients. TCGA analysis suggested CXCL17 expression was positively correlated with the transcriptional level of gastric mucosal maturation indicators TFF1, TFF2, PGC and MUC5AC, but negatively with the dedifferentiation indicators CDX1 and SFRP family members. Protein-protein interaction (PPI) revealed the potential connection of GPR35-CXCL17-CCL20. Overexpressing CXCL17 in HGC27 cell line downregulated SFRP2 and CDX1, but upregulated CCL20 expression.

Conclusion: CXCL17 and GPR35 may play a vital role in GC initiation and development, especially in the IM to GC process. A higher CXCL17 expression independently predicted for better survival in GC patients, with a possible role in maintaining gastric mucosa maturation.

Introduction

Gastric cancer (GC) is one of the most common cancers and its death rate ranks third among tumors worldwide¹. Patients with GC often have a poor prognosis because of low early diagnostic rate and limited therapeutic options². The initiation of GC is a stepwise process: under the synergistic effect of environmental and genetic elements, individuals sequentially develop gastric mucosal atrophy and/or intestinal metaplasia (IM), and GC. During this process, cell atypia degree gradually increases with the gradual accumulation of oncogenic molecular events³⁻⁵.

Chemokines are a group of small chemotactic cytokines known for their roles in recruiting and activating immunologic effectors. Evidence suggested that they play pivotal roles in inflammatory or immune related diseases and carcinogenesis^{6,7}. C-X-C motif chemokine 17 (CXCL17) is a newly characterized chemokine reported to be constitutively and inductively expressed in mucosal tissues and function in anti-inflammatory and anti-microorganism processes⁸⁻¹⁰. The role of CXCL17 in carcinogenesis probably depends on specific tumor context. Breast, hepatocellular, and colon cancers had high CXCL17 production, with a contribution to poor

survival; whereas CXCL17 exerted a protective role in pancreatic cancer through stimulating anti-tumor immune responses¹¹⁻¹⁵. Nevertheless, its involvement in GC initiation and development remains uncertain.

G-protein coupled receptor 35 (GPR35) is a member of the G protein-coupled receptors. These receptors are membrane proteins structurally characterized by seven membrane-spanning helices and responsible for transducing signaling induced by extracellular component change. In the report from José L. Maravillas-Montero et al., transfection of GPR35 into Ba/F3 cells, which are originally non-responsive to CXCL17 and don't express GPR35, could render them responsive to CXCL17¹⁶. Furthermore, downregulation of GPR35 was found in the lungs of *Cxcl17*^{-/-} mice in their study¹⁶. Based on these observations, GPR35 was defined as a possible receptor of CXCL17. Studies have associated GPR35 with several pathological processes like hypoxia, inflammation, pain transduction and tumorigenesis^{17,18}. The upregulation of GPR35 and its correlation with poor prognosis in lung and colon cancer tissues was reported^{11,19}. Activation of the GPR35 pathway can directly promote epithelial cell proliferation and coordinate macrophages' ability to create a tumor-permissive environment²⁰. It can also mediate chemoresistance partially via β -arrestin-2/Akt signaling and inhibition of this signaling could overcome chemoresistance¹⁹. However, its expression and involvement in gastric cancer initiation and development remains undefined.

In the present study, we detected the expression of CXCL17 and GPR35 in the normal gastric gland of non-atrophic gastritis (NAG-NOR), intestinal metaplasia of atrophic gastritis (AG-IM), intestinal metaplasia adjacent to GC (GC-IM), and GC tissues, aiming to reveal their expression during GC initiation and development and the clinical significance. We also explored the potential mechanism by which they participate in GC development.

Materials And Methods

Patients and tissue specimens

NAG-NOR (CXCL17, n = 30; GPR35, n = 30), AG-IM (CXCL17, n = 74; GPR35, n = 60), GC-IM (CXCL17, n = 88; GPR35, n = 91), and GC (CXCL17: n = 260; GPR35, n=227) tissues were collected from October 2012 to June 2019 in the First Affiliated Hospital of China Medical University. Samples were obtained from surgical operations or endoscopic biopsies. Patients with unavailable tissue samples or uncertain diagnosis were excluded. Clinical characteristics of patients were obtained from electronic medical record. Survival information was obtained from 111 patients in the CXCL17 group and 103 patients in the GPR35 group, respectively. The last follow-up date was November 25, 2019.

The study was approved by the Institute Research Medical Ethics Committee of the First Affiliated Hospital of China Medical University and informed consent was waived by the committee because of the difficulty for contacting the patients and that the patient's privacy was fully protected.

Immunohistochemistry

Formalin-fixed and paraffin-embedded gastric tissues were cut into 4- μ m-thick sections and subjected for immunohistochemistry using biotin-avidin complex method at room temperature. Briefly, after deparaffinizing and rehydrating, the sections were heated in boiled EDTA buffer for antigen retrieval. Then successive incubation

with primary and secondary antibodies was performed and DAB (DAB-0031, maxim Inc., Fujian, China) was used for staining. Anti-CXCL17 antibody (catalog number: MAB4207; R&D Systems, Inc.) was used at a concentration of 10 ug/ml and anti-GPR35 antibody (cytoplasmic domain ab 188949; Abcam) was used at a concentration of 7 ug/ml, respectively. For quality control, negative controls were run in parallel, and PBS was used instead of the primary antibody.

Evaluation of immunohistochemistry

Semi-quantitative scoring method was utilized to assess the expression level of CXCL17 and GPR35. The percentage of stained cells (S) and immunostaining intensity (I) were evaluated. The percentage score ranged from 0 to 4 (0% - 5% scores 0; 5%–25% scores 1; 25%–50% scores 2; 50%–75% scores 3; 75%–100% scores 4) and the intensity score ranged from 0 to 3 (0, no staining; 1, weak; 2, moderate; 3, strong). All fields under the microscope were viewed. Different pathological lesions were distinguished and scored by two experienced and highly qualified pathologists independently. The final IS score was obtained by multiplying the percentage of stained cells (S) with intensity (I), which ranged from 0 to 12.

TCGA data mining

GEPIA2 (<http://gepia2.cancer-pku.cn/#index>) website was used to compare the mRNA expression of CXCL17 and GPR35 in stomach adenocarcinoma (STAD) vs. corresponding normal tissues by matching TCGA normal and GTEx data²¹. R software (R 4.0.3) was applied for co-expression (volcano plots) and correlation analysis (correlation plot) utilizing TCGA data normalized by the log2 [TPM (Transcripts per million) +1] transformation. R package “limma” was used to identify the differential expression genes (DEGs) after dividing the TCGA samples into two groups according to the expression level of CXCL17 and GPR35, respectively. The DEGs shared by CXCL17 and GPR35 were used for PPI network construction using the “multiple protein” module of STRING, with the edges indicating both functional and physical protein associations (https://string-db.org/cgi/input?sessionId=bJl0ekoEqcJ1&input_page_show_search=on). Then the PPI result was import into Cytoscape software for visualization and disconnected nodes in the network were hidden.

Cell culture and transfection

HGC-27 (RRID: CVCL_1279) cell line was purchased from the cell bank of Chinese Academy of Medical Science (Beijing, China). It had been authenticated using STR profiling and were mycoplasma-free. Cells were cultured in RPMI 1640 medium (Solarbio, China) containing 10% foetal bovine serum (Biological industries, Israel) and placed in a 37°C incubator with 5% CO₂. For transfection, 60 to 80% confluent cells in a 6-well plate were transfected with CXCL17 overexpressing or empty plasmid (Gene, China) according to the jetPRIME® DNA transfection protocol. Twenty-four hours later, cells were lysed with TRIzol reagent (Tiangen, China) and the total RNA was extracted, reversed into cDNA and subjected to real-time fluorescence qPCR as indicated. β-Actin was applied for normalization. Program is set as 95 °C for 3min, and 45 cycles of 95 °C for 10s, 60 °C for 20s and 72 °C for 30s. The 2^{-ΔΔCT} method was adopted for calculation. The primer sequence of CXCL17 was as follows: forward 5'-TGCTGCCACTAATGCTGATGT-3' and reverse 5'-CTCAGGAACCAATCTTTGCACT-3'.

Western blot

Total proteins were isolated from cells 48 hours after transfection using RIPA Lysis Buffer (Beyotime) supplemented with protease inhibitors on ice for 30 min, following by centrifugation, quantification with a BCA protein assay kit (Beyotime) and denaturation by boiling. Then the proteins were separated by SDS-PAGE and transferred onto a PVDF membrane. The membrane was blocked with QuickBlock™ Blocking Buffer (Beyotime) for 15 minutes followed by incubation with primary antibodies overnight at 4°C. After washing with TBST for 3 times, the membrane was incubated with goat anti-rabbit or goat anti-mouse secondary antibodies (Abcam) at room temperature. The chemiluminescent signal was detected by an ECL method. The antibodies and dilutions applied in this study were CXCL17 (MAB4207, R&D): 1:1000, β -Tubulin (100109-MM05T, SinoBiological): 1:8000, CDX1 (204876-T08, SinoBiological): 1:2000, SFRP2 (ab92667, Abcam): 1:500 and CCL20 (10485-T24, SinoBiological): 1:2000.

Statistical analysis

All statistical analyses were conducted using SPSS software (version 26.0, Chicago, IL) or R software (R 4.0.3). Between-group comparisons of protein expression levels were analyzed by non-parametric test and quantitative data were presented as mean $\pm$ SD. The relationship between CXCL17 and GPR35 protein expressions was assessed by Spearman correlation analysis. Chi-square test was used to assess the associations of these two proteins' expressions with clinical parameters. Univariate survival analysis was performed by log-rank test and cox proportional hazards model was used for multivariate survival analysis adjusted by variables that had $P < 0.05$ in the univariate analysis (Supplemental Table S1). The median of IS score was used to identify the high and low expression groups. An IS score less than or equal to the median score was evaluated as low expression and higher than that score was identified as high expression. In cancerous cases, the median score was 3 and 6 in CXCL17 and GPR35 cohort, respectively. Reported P values were two-sided, and the significance level was set at 0.05 for all analyses.

Results

Expression of CXCL17 and GPR35 in different pathological lesions

The expression of CXCL17 in NAG-NOR vs. AG-IM was not significant ($P > 0.05$). CXCL17 expression was significantly higher in GC-IM than AG-IM or GC (both $P < 0.001$) (Fig.1A). Likewise, the expression of GPR35 in NAG-NOR vs. AG-IM was not significant ($P > 0.05$), while it was significantly upregulated in GC-IM in comparison to AG-IM or GC ($P < 0.001$ and $P < 0.01$, respectively) (Fig.1B). The representative immunostaining pictures were displayed in Fig.1C, with CXCL17 mainly expressed in the cytoplasm of gastric epithelial cells and GPR35 in the cell membrane of GC-IM and GC cells.

Correlation between CXCL17 and GPR35 expressions

The expression correlation between CXCL17 and GPR35 was assessed according to different pathological lesions (Fig.2). They had a significantly positive correlation in GC tissues (Spearman $r = 0.2933$, $P < 0.001$) (Fig.2A) rather than in the NAG-NOR, AG-IM or GC-IM tissues (each P value > 0.05) (Fig.2B-D).

Clinical significance of CXCL17 and GPR35 expressions in GC

Clinical information, including gender, age, *H.pylori* infection status, tumor location, histological type, tumor stage, the presence of lymph node metastasis, perineural invasion, and vessel carcinoma embolus, and tumor growth pattern, on 141 patients in the CXCL17 group and 134 patients in the GPR35 group were obtained, respectively (Table 1). Incomplete data were noted with superscript. CXCL17 expression was negatively related to tumor invasive depth ($P = 0.015$) and maximum diameter ($P = 0.003$). Infiltrative growth pattern was more common in GC cases whose tumor tissues lowly expressed CXCL17. The expression level of GPR35 was associated with age and tumor histological types: patients over 60 years old or whose tumors were adenocarcinoma tended to have higher GPR35 expression. No significance was shown for other clinical parameters.

Prognostic significance of CXCL17 and GPR35 expressions in GC

111 and 103 GC cases were included for prognostic analysis in the CXCL17 and GPR35 groups, respectively. Patients with higher CXCL17 expressions had better survival in comparison to those with lower expressions (median OS: not reached vs. 31 months; log-rank $P = 0.031$) (Fig 3A). The median OS of patients with high GPR35 expression was 42 months, which was longer than the 36 months of patients with low GPR35 expression, but the difference was not significant ($P = 0.616$) (Fig 3B). Besides, multivariate survival analysis suggested that the high expression of CXCL17 independently predicted for a better overall survival in GC patients ($P = 0.027$; HR = 0.484, 95%CI = 0.255-0.919) (Table 2).

Identification of DEGs by TCGA analysis

Comparison of the transcriptional levels of CXCL17 and GPR35 in stomach adenocarcinoma (STAD) vs. corresponding normal tissues exhibited that CXCL17 was downregulated while GPR35 was upregulated in STAD (Fig.4A and 4B).

TCGA samples were divided into two groups according to the expression level of CXCL17 and GPR35, respectively. DEGs were identified based on $|\log FC| \geq 0.5$ and adjust P value ≤ 0.05 (Fig 4C and 4D). The top 20 positively or negatively regulated genes were visualized in the correlation plot (Fig.4E and 4F). The expression of CDX1 was negatively correlated with CXCL17 but positively correlated with GPR35; SFRP2 expression was negatively correlated with both CXCL17 and GPR35. Notably, CXCL17 expression was positively correlated with the expression of gastric mucosal maturation indicators TFF1, TFF2, PGC and MUC5AC, but negatively correlated with the dedifferentiation indicators CDX1 and SFRP family members, suggesting the involvement of CXCL17 in gastric cell differentiation and its potential contribution to gastric epithelial cell maturation.

PPI network construction

The DEGs shared by CXCL17 and GPR35 were used for PPI network construction and the results showed that CXCL17, GPR35 and CCL20 might be functionally associated (Fig.5A and 5B).

Regulatory roles of CXCL17 in HGC27 cell line

Given that CXCL17 may contribute to GC differentiation, we selected the poorly differentiated GC cell line HGC27 for further validation by Cancer Cell Line Encyclopedia (CCLE) analysis (Fig 6A). The fluorescent photograph (Fig.6B), qPCR (Fig.6C) and western blot (Fig.6D) verified the successful overexpression of CXCL17 in HGC27 cell line. Overexpressing CXCL17 significantly downregulated the expression of CDX1 and SFRP2 (Fig.6D). CCL20 expression was significantly upregulated after overexpressing CXCL17 (Fig.6D).

Discussion

CXCL17 is a newly discovered mucosal chemokine known to recruit macrophages and likely to play both a harmful and protective role in human health and disease²². Different types of cancer may utilize CXCL17 in totally distinct ways. Nevertheless, the expression pattern of CXCL17 during GC initiation and development, in other words, the dynamic process of gastric lesion aggravation, remains undefined. We detected the protein expression of CXCL17 and its possible receptor, GPR35, in NAG-NOR, AG-IM, GC-IM, and GC tissues, and evaluated their clinical significance as well as prognostic value. The potential regulatory and biological roles of CXCL17 were further explored through TCGA data mining and *in vitro* experiment.

GC seldom develops from the normal gastric mucosa, but where originally lies a pathological lesion, such as the precancerous lesion IM. Most GCs had experienced the process of IM before the occurrence of gastric cancer. We found that CXCL17 expression gradually increased from NAG-NOR, AG-IM to GC-IM, but dramatically downregulated in GC tissues. This implied the loss of CXCL17 may be a key molecular event contributing to GC development. A prior study evaluated the expression of CXCL17 in 22 different rat tissues and found it had highest expression in stomach; furthermore, treating the primary gastric cells isolated from rat glandular stomachs with bacteria-derived LPS significantly increased CXCL17 expression in a dose-dependent manner⁹. These suggested CXCL17 exhibited stable gastric mucosa expression under physiological conditions. Stimuli like bacterial materials can promote its expression probably for resisting harmful responses. Consistent with the above conjecture, we observed the frequent and sequentially increased expression of CXCL17 in NAG-NOR, AG-IM and GC-IM tissues. We speculate that this was also the result of cells stimulated by gastric environment during the development of gastric lesions and may protect gastric cells from cancer cell transformation.

Clinically, low expression of CXCL17 indicated deeper invasive extent, tendency of infiltrative tumor growth pattern, and larger tumor volume as suggested from our analysis. The low expression also independently predicted for better prognosis for GC patients. These results supported our speculation that CXCL17 may play an inhibitory role during GC development, though the underlying mechanism needs further studies to clarify.

Studies have identified the upregulation of GPR35 in lung and colon cancers and its association with poor prognosis^{19,23}. A more recent study reported the dual role of GPR35 in promoting colon cancer, where it could

directly augment proliferation of epithelial cells, and facilitated tumor-permissive environment formation via macrophages²⁰. Nevertheless, its expression in different gastric lesions and clinical significance in GC needed study to illustrate. We ascertained the absent expression of GPR35 in NAG-NOR and AG-IM, and high expression in GC-IM and GC. This result proved that the upregulation of GPR35 was initiated in the process of IM to GC. Additionally, GC tissues exhibited significantly lower expression of GPR35 in comparison to GC-IM. Combining these results, we speculated GPR35 exhibited a two-stage regulation pattern during GC initiation and development and a more complex role which needs to be proved by further in-depth experiments.

The binding of CXCL17-GPR35 was identified due to the ability of GPR35 to render responsibility to CXCL17 in Ba/F3 cells and their similar expression patterns in mucosa tissues¹⁶. However, two later studies showed the specificity of their binding was not strong and there might be other receptors distinct from GPR35^{24,25}. We evaluated the correlation between the expressions of CXCL17 and GPR35 in different pathological lesions. The strength of association observed here (a spearman r of 0.2933) in GC tissues was similar to that reported (a spearman r of 0.295) in colon cancer¹³. However, the pre-cancerous lesions didn't exhibit this association. These results may indicate that GPR35 was probably not a bona fide receptor for CXCL17, as suggested by Nurul A S Binti Mohd Amir et al²⁴. And this might also indicate that the binding of CXCL17 to GPR35 was not strong, and of tissue specific, which of course, required further research confirmation.

To further explore the potential roles of CXCL17 and GPR35 in GC development, we performed TCGA data mining and preliminary concluded that CXCL17 may be associated with the process of gastric mucosal epithelium maturation based on the observation that its expression was positively associated with TFF1, TFF2, PGC and MUC5AC expressions, and negatively associated with CDX1 and SFRP family members expressions. TFF1, TFF2 and MUC5AC were proved to play an important role in maintaining the integrity and barrier function of gastric mucosa against bacterial colonization and the development of chronic inflammation²⁶⁻²⁹. Their loss of expression is closely related to the occurrence of GC^{30,31}. We suggested that CXCL17 may have a similar effect on gastric mucosa. CDX1 is a DNA binding protein related to the intestinal phenotype of gastric epithelial cells and may also be involved in the development of *Hp*-related GC^{32,33}. SFRP2, whose full name is secreted frizzled related protein 2, can directly bind to Wnts to regulate Wnt signaling participating in cell growth and differentiation³⁴⁻³⁷. On the basis of TCGA data mining, we verified that overexpressing CXCL17 in HGC27, a poorly differentiated GC cell line, downregulated the expression of CDX1 and SFRP2. The regulation of CXCL17 on CDX1 and SFRP2 may be one of the possible mechanisms by which CXCL17 participates in the differentiation of gastric epithelial cells. In addition, we found that overexpressing CXCL17 significantly upregulate CCL20, a chemokine with chemotaxis to dendritic cells and regulatory T cells. Like CXCL17, CCL20 was reported to play an important role in mucosal immunity in homeostasis or inflammation status³⁸⁻⁴⁰. Our results suggest that CXCL17 and CCL20 may have a synergistic effect.

In conclusion, CXCL17 and GPR35 may play a vital role in GC initiation and development, especially in the IM to GC process. A higher CXCL17 expression independently predicted for better survival in GC patients, with a possible role in maintaining gastric mucosa maturation.

Declarations

Authors' contributions

Yuan Yuan and Qian Xu conceived and designed this study. Yizhi Li performed the experiments, data analyses and wrote the manuscript. Aoran Liu contributed significantly to the experiment and data analysis. Songyi Liu and Lirong Yan helped perform the analysis with constructive discussions. Qian Xu and Yuan Yuan revised the manuscript.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Acknowledgements

Not applicable.

Funding

This work was supported by the National Key R&D Program of China (2017YFC0907402)

Data availability statement

The data that support the findings of our study are available upon request from the corresponding author.

Ethics statement

The study was approved by the Institute Research Medical Ethics Committee of the First Affiliated Hospital of China Medical University and informed consent was waived by the committee because of the difficulty for contacting the patients and the patient's privacy was fully protected.

Consent for publication

Not applicable.

References

1. Thrift AP, El-Serag HB. Burden of Gastric Cancer. *Clin Gastroenterol Hepatol*.2020;18(3):534-542.
2. Smyth EC, Nilsson M, Grabsch HI, van Grieken NC, Lordick F. Gastric cancer. *Lancet*.2020;396(10251):635-648.
3. Raza M, Bhatt H. Atrophic Gastritis. In: *StatPearls*. Treasure Island (FL)2020.
4. Piazzuelo MB, Riechelmann RP, Wilson KT, Algood HMS. Resolution of Gastric Cancer-Promoting Inflammation: A Novel Strategy for Anti-cancer Therapy. *Curr Top Microbiol Immunol*.2019;421:319-359.

5. Koliaraki V, Prados A, Armaka M, Kollias G. The mesenchymal context in inflammation, immunity and cancer. *Nat Immunol.*2020;21(9):974-982.
6. Mollica Poeta V, Massara M, Capucetti A, Bonecchi R. Chemokines and Chemokine Receptors: New Targets for Cancer Immunotherapy. *Front Immunol.*2019;10:379.
7. Nagarsheth N, Wicha MS, Zou W. Chemokines in the cancer microenvironment and their relevance in cancer immunotherapy. *Nat Rev Immunol.*2017;17(9):559-572.
8. Xiao S, Xie W, Zhou L. Mucosal chemokine CXCL17: What is known and not known. *Scand J Immunol.*2020:e12965.
9. Lee WY, Wang CJ, Lin TY, Hsiao CL, Luo CW. CXCL17, an orphan chemokine, acts as a novel angiogenic and anti-inflammatory factor. *Am J Physiol Endocrinol Metab.*2013;304(1):E32-40.
10. Srivastava R, Hernandez-Ruiz M, Khan AA, et al. CXCL17 Chemokine-Dependent Mobilization of CXCR8(+)CD8(+) Effector Memory and Tissue-Resident Memory T Cells in the Vaginal Mucosa Is Associated with Protection against Genital Herpes. *J Immunol.*2018;200(8):2915-2926.
11. Guo YJ, Zhou YJ, Yang XL, Shao ZM, Ou ZL. The role and clinical significance of the CXCL17-CXCR8 (GPR35) axis in breast cancer. *Biochem Biophys Res Commun.*2017;493(3):1159-1167.
12. Li L, Yan J, Xu J, et al. CXCL17 expression predicts poor prognosis and correlates with adverse immune infiltration in hepatocellular carcinoma. *PLoS One.*2014;9(10):e110064.
13. Yao H, Lv Y, Bai X, Yu Z, Liu X. Prognostic value of CXCL17 and CXCR8 expression in patients with colon cancer. *Oncol Lett.*2020;20(3):2711-2720.
14. Wang L, Li H, Zhen Z, et al. CXCL17 promotes cell metastasis and inhibits autophagy via the LKB1-AMPK pathway in hepatocellular carcinoma. *Gene.*2019;690:129-136.
15. Hiraoka N, Yamazaki-Itoh R, Ino Y, et al. CXCL17 and ICAM2 are associated with a potential anti-tumor immune response in early intraepithelial stages of human pancreatic carcinogenesis. *Gastroenterology.*2011;140(1):310-321.
16. Maravillas-Montero JL, Burkhardt AM, Hevezi PA, Carnevale CD, Smit MJ, Zlotnik A. Cutting edge: GPR35/CXCR8 is the receptor of the mucosal chemokine CXCL17. *J Immunol.*2015;194(1):29-33.
17. Schneditz G, Elias JE, Pagano E, et al. GPR35 promotes glycolysis, proliferation, and oncogenic signaling by engaging with the sodium potassium pump. *Sci Signal.*2019;12(562).
18. Divorty N, Mackenzie AE, Nicklin SA, Milligan G. G protein-coupled receptor 35: an emerging target in inflammatory and cardiovascular disease. *Front Pharmacol.*2015;6:41.
19. Wang W, Han T, Tong W, Zhao J, Qiu X. Overexpression of GPR35 confers drug resistance in NSCLC cells by beta-arrestin/Akt signaling. *Onco Targets Ther.*2018;11:6249-6257.
20. Pagano E, Elias JE, Schneditz G, et al. Activation of the GPR35 pathway drives angiogenesis in the tumour microenvironment. *Gut.*2021.
21. Tang Z, Kang B, Li C, Chen T, Zhang Z. GEPIA2: an enhanced web server for large-scale expression profiling and interactive analysis. *Nucleic Acids Res.*2019;47(W1):W556-W560.
22. Chorenó-Parra JA, Thirunavukkarasu S, Zuniga J, Khader SA. The protective and pathogenic roles of CXCL17 in human health and disease: Potential in respiratory medicine. *Cytokine Growth Factor Rev.*2020;53:53-62.

23. Ali H, AbdelMageed M, Olsson L, et al. Utility of G protein-coupled receptor 35 expression for predicting outcome in colon cancer. *Tumour Biol.*2019;41(6):1010428319858885.
24. Binti Mohd Amir NAS, Mackenzie AE, Jenkins L, et al. Evidence for the Existence of a CXCL17 Receptor Distinct from GPR35. *J Immunol.*2018;201(2):714-724.
25. Park SJ, Lee SJ, Nam SY, Im DS. GPR35 mediates Iodoxamide-induced migration inhibitory response but not CXCL17-induced migration stimulatory response in THP-1 cells; is GPR35 a receptor for CXCL17? *Br J Pharmacol.*2018;175(1):154-161.
26. Esposito R, Morello S, Vllahu M, Eletto D, Porta A, Tosco A. Gastric TFF1 Expression from Acute to Chronic Helicobacter Infection. *Front Cell Infect Microbiol.*2017;7:434.
27. Soutto M, Chen Z, Bhat AA, et al. Activation of STAT3 signaling is mediated by TFF1 silencing in gastric neoplasia. *Nat Commun.*2019;10(1):3039.
28. Perez-Vilar J, Randell SH, Boucher RC. C-Mannosylation of MUC5AC and MUC5B Cys subdomains. *Glycobiology.*2004;14(4):325-337.
29. Van de Bovenkamp JH, Mahdavi J, Korteland-Van Male AM, et al. The MUC5AC glycoprotein is the primary receptor for Helicobacter pylori in the human stomach. *Helicobacter.*2003;8(5):521-532.
30. Hoffmann W. TFF2, a MUC6-binding lectin stabilizing the gastric mucus barrier and more (Review). *Int J Oncol.*2015;47(3):806-816.
31. Heuer F, Sturmer R, Heuer J, et al. Different Forms of TFF2, A Lectin of the Human Gastric Mucus Barrier: In Vitro Binding Studies. *Int J Mol Sci.*2019;20(23).
32. Choi SI, Yoon C, Park MR, et al. CDX1 Expression Induced by CagA-Expressing Helicobacter pylori Promotes Gastric Tumorigenesis. *Mol Cancer Res.*2019;17(11):2169-2183.
33. Fujii Y, Yoshihashi K, Suzuki H, et al. CDX1 confers intestinal phenotype on gastric epithelial cells via induction of stemness-associated reprogramming factors SALL4 and KLF5. *Proc Natl Acad Sci U S A.*2012;109(50):20584-20589.
34. Zhao B, Chen Y, Yang N, et al. miR-218-5p regulates skin and hair follicle development through Wnt/beta-catenin signaling pathway by targeting SFRP2. *J Cell Physiol.*2019;234(11):20329-20341.
35. Han M, Wang S, Fritah S, et al. Interfering with long non-coding RNA MIR22HG processing inhibits glioblastoma progression through suppression of Wnt/beta-catenin signalling. *Brain.*2020;143(2):512-530.
36. Wu J, Li H, Shi M, et al. TET1-mediated DNA hydroxymethylation activates inhibitors of the Wnt/beta-catenin signaling pathway to suppress EMT in pancreatic tumor cells. *J Exp Clin Cancer Res.*2019;38(1):348.
37. Heinosaalo T, Gabriel M, Kallio L, et al. Secreted frizzled-related protein 2 (SFRP2) expression promotes lesion proliferation via canonical WNT signaling and indicates lesion borders in extraovarian endometriosis. *Hum Reprod.*2018;33(5):817-831.
38. Wang D, Yang L, Yu W, et al. Colorectal cancer cell-derived CCL20 recruits regulatory T cells to promote chemoresistance via FOXO1/CEBPB/NF-kappaB signaling. *J Immunother Cancer.*2019;7(1):215.
39. Sebrell TA, Hashimi M, Sidar B, et al. A Novel Gastric Spheroid Co-culture Model Reveals Chemokine-Dependent Recruitment of Human Dendritic Cells to the Gastric Epithelium. *Cell Mol Gastroenterol Hepatol.*2019;8(1):157-171 e153.

40. Ito T, Carson WFt, Cavassani KA, Connett JM, Kunkel SL. CCR6 as a mediator of immunity in the lung and gut. *Exp Cell Res.*2011;317(5):613-619.

Tables

Table 1

Associations of CXCL17 and GPR35 protein expressions with clinicopathological parameters in GC.

Variables	CXCL17 expression				GPR35 expression			
	Cases	Low n(%)	High n(%)	P value	Cases	Low n(%)	High n(%)	P value
Total	141				134	74	60	
Gender								
Male	108	61(56.5)	47(43.5)		99	56(56.6)	43(43.4)	
Female	33	23(69.7)	10(30.3)	0.176	35	18(51.4)	17(48.6)	0.599
Age(years)								
>60	76	40(52.6)	36(47.4)		71	33(46.5)	38(53.5)	
<=60	65	44(67.7)	21(32.3)	0.069	63	41(65.1)	22(34.9)	0.031
<i>H.pylori</i> infection ¹								
Positive	71	43(60.6)	28(39.4)		63	36(57.1)	27(42.9)	
Negative	30	20(66.7)	10(33.3)	0.563	31	14(45.2)	17(54.8)	0.274
Tumor location								
Body only	22	11(50.0)	11(50.0)		22	12(54.5)	10(45.5)	
Antrum only	73	42(57.5)	31(42.5)		72	37(51.4)	35(48.6)	
Others	46	31(67.4)	15(32.6)	0.345	40	25(62.5)	15(37.5)	0.525
Histological type								
Adenocarcinoma	112	65(58.0)	47(42.0)		104	52(50.0)	52(50.0)	
Absolute signet ring cell carcinoma	8	6(75.0)	2(25.0)		8	8(100.0)	0(0.0)	
Mixed carcinoma	19	11(57.9)	8(42.1)		20	13(65.0)	7(35.0)	
Lymphoepithelioma-Like Carcinoma	2	2(100.0)	0(0.0)	0.516	2	1(50.0)	1(50.0)	0.038
Stage								
Early	9	4(44.4)	5(55.6)		10	8(80.0)	2(20.0)	
Advanced	132	80(60.6)	52(39.4)	0.485	124	66(53.2)	58(46.8)	0.184
Invasive extent ¹								
T1	10	3(30.0)	7(70.0)		11	8(72.7)	3(27.3)	
T2	23	10(43.5)	13(56.5)		19	10(52.6)	9(47.4)	
T3	17	8(47.1)	9(52.9)		17	9(52.9)	8(47.1)	

T4	90	62(68.9)	28(31.1)	0.015	86	46(53.5)	40(46.5)	0.671
TNM stage1								
I	29	17(58.6)	12(41.4)		28	16(57.1)	12(42.9)	
II	30	16(53.3)	14(46.7)		28	15(53.6)	13(46.4)	
III	79	50(63.3)	29(36.7)		75	40(53.3)	35(46.7)	
IV	1	0(0.0)	1(100.0)	0.489	1	1(100.0)	0(0.0)	0.810
Lymph node metastasis1								
Positive	98	61(62.2)	37(37.8)		93	51(54.8)	42(45.2)	
Negative	40	21(52.5)	19(47.5)	0.290	38	21(55.3)	17(44.7)	0.965
Perineural invasion ¹								
Positive	113	69(61.1)	44(38.9)		110	57(51.8)	53(48.2)	
Negative	25	12(48.0)	13(52.0)	0.230	20	14(70.0)	6(30.0)	0.133
Vessel carcinoma embolus1								
Positive	85	51(60.0)	34(40.0)		79	41(51.9)	38(48.1)	
Negative	55	32(48.0)	23(41.8)	0.831	54	32(59.3)	22(40.7)	0.402
Growth pattern								
superficial spreading	2	1(50.0)	1(50.0)		3	3(100.0)	0(0.0)	
Infiltrative	118	76(64.4)	42(35.6)		112	64(57.1)	48(42.9)	
Nested/cloddy	21	7(33.3)	14(66.7)	0.027	19	7(36.8)	12(63.2)	0.074
Maximum diameter(cm)1								
>4.5	84	58(69.0)	26(31.0)		83	42(50.6)	41(49.4)	
<=4.5	55	24(43.6)	31(56.4)	0.003	49	31(63.3)	18(36.7)	0.157

¹ Incomplete information

Table 2

Univariate and multivariate analysis of survival in gastric cancer patients

Variables	Cases	Univariable Analysis	Multivariable Analysis	
		P value	Hazard Ratio (95% CI)	P value
CXCL17 expression (Low vs. high)	111	0.031	0.484(0.255-0.919)	0.027
GPR35 expression (Low vs. high)	103	0.616	1.005(0.563-1.794)	0.986
TNM stage (I+II vs. III+IV)	111	0.005	1.787(0.917-3.481)	0.088
Perineural invasion (No vs. yes)	111	0.003	4.938(1.156-21.086)	0.031
Vessel carcinoma embolus (No vs. yes)	111	0.047	1.073(0.557-2.065)	0.834
Maximum diameter (<=4.5 vs. >4.5cm)	111	0.008	1.865(0.892-3.897)	0.098

Invasive extent and lymph node metastasis were not included for analysis because they are closely related to TNM stage

Figures

Figure 1

The protein expression of CXCL17 and GPR35 in NAG-NOR, AG-IM, GC-IM and GC. A. Comparison of CXCL17 expression; B. Comparison of GPR35 expression; C. Representative images of CXCL17 and GPR35 immunostaining, respectively. Magnification: $\times 200$. Abbreviations: NAG-NOR, normal gastric gland of non-atrophic gastritis; AG-IM, intestinal metaplasia of atrophic gastritis; GC-IM, intestinal metaplasia adjacent to gastric cancer; GC, gastric cancer.

Figure 2

Correlation between CXCL17 and GPR35 expressions. A. GC; B. GC-IM; C. AG-IM; D. NAG-NOR. Abbreviations: NAG-NOR, normal gastric gland of non-atrophic gastritis; AG-IM, intestinal metaplasia of atrophic gastritis; GC-IM, intestinal metaplasia adjacent to gastric cancer; GC, gastric cancer.

Figure 3

CXCL17 and GPR35 expressions with GC prognosis. A. K-M curve for CXCL17; B. K-M curve for GPR35

Figure 4

Identification of DEGs by TCGA analysis. A. The mRNA level of CXCL17 and GPR35 in STAD vs. normal tissues; B. The mRNA level of GPR35 in STAD vs. normal tissues; C. Volcano plot of the DEGs in CXCL17-high vs. CXCL17-low; D. Volcano plot of the DEGs in GPR35-high vs. GPR35-low; Red color is indicative of upregulated genes and green color of downregulated genes; E. Correlation plot of the top 20 up- and down-regulated genes of CXCL17; F. Correlation plot of the top 20 up and down-regulated genes for GPR35; Red dots indicate positive correlations and blue dots indicate negative correlations. Abbreviations: DEGs, differentially expressed genes.

Figure 5

PPI construction. A. Eighty-nine genes were identified shared by CXCL17 and GPR35; B. These eighty-nine genes were used for PPI construction following visualization by Cytoscape with disconnect nodes discarded. Abbreviations: PPI, protein-protein interaction

Figure 6

TCGA validation. A. CXCL17 expression in different gastric cell lines by CCLE analysis; B. Fluorescent photograph after transfection of CXCL17 into HGC27 cell line; C. The relative mRNA expression of CXCL17 in CXCL17 CON vs. CXCL17 OE after CXCL17 transfection; D. The regulatory role of CXCL17 on CDX1, SFRP2 and CCL20 in HGC27 cells. Abbreviations: CCLE, Cancer Cell Line Encyclopedia; CON, control; OE, overexpression

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [TableS1.docx](#)