Unique membranous gastrin receptor expression of parietal cells and its distribution pattern in oxyntic gastric mucosa and fundic gland polyps

Short running title: Unique gastrin receptor expression in the stomach

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Summary

The aim of this study was to clarify the gastrin receptor (GR) expression in gastric oxyntic gland mucosa and fundic gland polyps (FGPs), especially focusing on the GR expression in parietal cells, and to correlate the GR expression and the organization status of the mucosa, considering the PPI medication history. On immunohistochemistry, the unique membranous linear positivity of GR in parietal cells was reproducibly observed, which was also validated by immunofluorescence. For further evaluation, samples of 34 oxyntic gland mucosae and 43 FGPs were histologically and immunohistochemically analyzed and correlated with demographic/clinical information of the patients. The parietal cells with membranous linear GR expression seemed to be limited to the isthmus-neck region in normal state, and their appearance in deep oxyntic gland areas was significantly related to the medication history of PPI, and was more frequently observed in the oxyntic gland mucosa with deranged component cell organization revealed by MUC5AC and MUC6 immunohistochemistry, which was also significantly related to the PPI use. With regard to FGPs, the parietal cells with intense membranous linear positivity of GR were observed and distributed diffusely in all of the cases. In conclusion, the present study revealed the unique GR membranous linear expression in parietal cells. Its distribution mode in oxyntic gland mucosa and FGPs could be related to deranged gland architecture, suggesting that the GR expression would play a role in maintaining the mucosal organization.

Keywords: gastrin receptor; oxyntic gland mucosa; fundic gland polyp; proton pump inhibitor; immunohistochemistry

1. Introduction

Gastrin receptor (GR), also known as cholecystokinin B receptor (CCKBR), is a G protein-coupled receptor for gastrin and cholecystokinin B. Human GR is distributed in the stomach, pancreas, and central nervous system, which is involved in a variety of physiological aspects [1]. In the stomach, the expression of GR is reportedly found in parietal cells and enterochromaffin-like cells (ECL cells) of the oxyntic gland region [2,3]. Gastrin, the ligand of GR, is released by gastric G cells located in the junctional region of the foveola and pyloric glands [4]. Gastrin stimulates secretion of gastric acid, either directly by binding to the GR of parietal cells, or indirectly by enhancing histamine release from ECL cells [5,6]. Besides, the Gastrin-GR system is known to promote the proliferation of ECL cells by activating the MAP kinase pathway [5,7,8], and to stimulate gastric mucosal epithelial growth by releasing several growth factors from ECL cells and parietal cells [9-11]. GR has also been reported to be expressed in several human carcinomas and tumors including the gastric origin [12,13]. Thus, the Gastrin-GR system would be involved in physiological and pathophysiological functions beyond just gastric acid secretion.

Recently, proton pump inhibitors (PPIs) have been widely medicated for various clinical settings such as gastroesophageal reflux diseases. These potent gastric acid suppressors have also been known to cause structural and functional change of the gastric mucosa component cells such as parietal cells, foveolar cells, and neuroendocrine cells, including the formation of fundic gland polyps (FGPs) [14,15], in which the Gastrin-GR system is considered to be involved and to play a role.

In these situations, the mode of GR expression in human oxyntic gland mucosa has been studied in several reports [3,16]. However, it does not seem to be fully established in connection with the status of the mucosa organization, nor the alteration of the GR expression caused in relation to PPI use. Therefore, in this study, we revaluated and confirmed the GR expression in gastric oxyntic gland mucosa and FGPs, especially focusing on the GR expression in parietal cells, and correlated the GR expression and the organization status of the mucosa, considering the PPI medication history.

2. Materials and methods

2.1. Patients and samples

This retrospective study was conducted with the approval of the Ethics Committee of Dokkyo Medical University Saitama Medical Center (Approval number: 19102).

The oxyntic gland mucosae for the analyses were obtained from the specimens which had been surgically or endoscopically resected for gastric adenocarcinoma, carcinoma originating in esophago-gastric junction, esophageal carcinoma or gastrointestinal stromal tumor. 34 samples from 34 patients were enrolled in the study. Each sample was selected after the histological review confirming that it contained “normal oxyntic gland mucosa” without remarkable changes such as obvious mucosal atrophy, severe inflammation, dysplastic change or intestinal metaplasia. As for fundic gland polyp (FGP), 43 lesions from 33 patients were selected and enrolled in the study after histological review of the samples confirming the findings consistent with

FGP. The samples of these lesions were obtained by biopsy or endoscopic mucosal resection on esophagogastroduodenoscopy. All of the above were specimens and samples submitted to and pathologically diagnosed at Department of Pathology, Dokkyo Medical University Saitama Medical Center, Koshigaya, Japan, during the period from the year of 2013 to 2021.

The demographic and clinical features of the cases were obtained from their medical records, focusing on age, sex, the location from which the samples were obtained in the stomach, the medication history of proton pump inhibitors (PPIs) and/or Histamine H2-receptor antagonists, and the diameter of FGPs. The location in the stomach was categorized according to the designation in Japanese Classification of Gastric Carcinoma (the 15th edition, October 2017) as U, M, and L [17].

2.2. Histological preparation and immunohistochemistry

Hematoxylin and eosin staining (HE) using the routine protocol at our department and immunohistochemistry (IHC) were performed on 10% buffered formalin-fixed paraffin-embedded (FFPE) sections. Immunohistochemical procedures for MUC5AC, MUC6, and Ki-67 were performed by Dako Autostainer Link 48 (Agilent Technologies, Santa Clara, CA, USA) according to the manufacturer's instructions, using the primary antibodies as follows: anti-MUC5AC (clone CLH2, Leica, Newcastle, UK), anti-MUC6 (clone CLH5, Leica, Newcastle, UK), and anti-Ki-67 (GA62661-2, DAKO omnis, Glostrup, Denmark). 3,3'-diaminobenzidine tetrahydrochloride (DAB) was used as chromogen.

The IHC of GR was performed manually using the primary antibody against

CCKBR (1:200; EB06767, Everest Biotech, UK). The Envision+ System HRP-labelled polymer (DAKO) with DAB as chromogen was used for the development of the staining. GR protein absorption test was successfully performed using the immunizing peptide (EBP06767; Everest Biotech, IgG: peptide = 1: 20) to ensure the specificity of GR IHC result (Supplementary Fig. S1). Besides, to validate the results of the above IHC of GR, the detection of GR protein was again performed by immunofluorescence (IF) using the same primary antibody for GR (1:100; EBP06767, Everest Biotech) on FFPE sections of the selected cases of the oxyntic gland mucosa (Supplementary of the methods in detail).

2.3. In situ hybridization

In situ hybridization (ISH) assay was performed on FFPE sections of several selected cases of both oxyntic gland mucosa and FGP by using ISH RNAscope® 2.5 HD Assay-Brown, according to the protocols provided by Advanced Cell Diagnostics (ACD, CA, USA) with a few modifications (Supplementary of the methods in detail) [18].

2.4. Analyses of histological and immunohistochemical findings

For all of the samples examined, histological features on HE preparation were checked focusing on the presence of hyperplasia-like change in the superficial foveolar epithelium (foveolar cell hyperplasia-like change), parietal cell change including apocrine secretion-like cytoplastic protrusion (parietal cell protrusion) and microvesicular cytoplasmic vacuolation (parietal cell vacuolation), and moderate or a higher degree of mononuclear inflammatory cell infiltration based

on the Updated Sydney System [19]. In addition, for each lesion of FGP, the maximum diameter of the cystic glands was measured (Figure 6A, B). Other semiquantitative and quantitative evaluations of histological and immunohistochemical findings were also carried out as described below in Results. The measurement of on the HE and IHC preparation were digitally performed using an imaging software (cellSens Standard 1.8.1, OLYMPUS, Tokyo, Japan). Every Ki-67 positive rate (%) in the selected areas at a high magnification (objective lens ×400) was counted and calculated by a specialized software e-Count2 (e-Path digital pathology, Fujisawa, Japan).

2.5. Statistical analysis

Fisher's exact test, the Mann–Whitney U-test, and Pearson's correlation analysis were performed, using the freely available statistic software EZR version 1.54 (Division of Hematology, Saitama Medical Center, Jichi Medical University, Saitama, Japan) [20]. A value of $P < 0.05$ was set to be considered significant.

3. Results

3.1. Detection and validation of GR expression in oxyntic gland mucosa

The immunohistochemical analysis of GR in the oxyntic gland mucosa revealed membranous linear positivity in addition to weak cytoplasmic staining in parietal cells (Fig. 1A and 1B). The membranous linear positivity was consistently limited to the luminal side of the cells. This linear expression pattern was reproducibly observed in most of the examined cases of oxyntic gland mucosa, which was also confirmed by the immunofluorescence study (Fig. 1C). To validate

the expression of GR in the mRNA level, an ISH assay for the mRNA of GR was conducted, confirming that the dot-like positive signals were observed in the parietal cells (Fig. 1D). There were no distinctive differences in the ISH results among cases with different GR distribution patterns on the IHC as defined below.

3.2. Categorization of GR expression patterns in oxyntic gland mucosa

Based on the results of the IHC, a tendency was noticed that the parietal cells showing the membranous linear GR positivity were often distributed in limited areas of oxyntic gland mucosa, while weak cytoplasmic positivity of GR was observed for all parietal cells. Therefore, we categorized the GR distribution according to the immunohistochemical membranous linear positivity of parietal cells into four patterns (Pattern 0 to 3) as shown in Fig. 2 (As for the intensity of GR positivity, see Supplementary of the results and Fig. S2).

3.3. Correlation between GR expression and demographic/clinical features of patients, or histological alterations of oxyntic gland mucosa

As for the GR distribution patterns defined above, we could divide them into two groups with or without involvement of the base region by the parietal cells showing membranous linear GR expression, that is, Pattern (0, 1) group and Pattern (2, 3) group.

Analyzing the correlation between these two groups and the demographic/clinical variables of the patients, only the medication history of PPIs was significantly correlated (P=0.034), showing more frequent Pattern (2, 3) group in the patients with PPI medication than in the patients without PPI

medication (Table 1). On the other hand, the histological features focused here were not correlated with the GR expression patterns (Table 1).

3.4. Status of component cell compartmentalization in oxyntic gland mucosa, and its correlation with GR expression or PPI use

As shown in Fig. 3A, the epithelial cell components of intact oxyntic gland mucosa are well-organized and compartmentalized from the surface to the bottom, the derangement of which was observed in various degrees in the examined oxyntic gland mucosae histologically and immunohistochemically. To evaluate the status of component cell compartmentalization in oxyntic gland mucosa, we set several indicators as defined in Fig. 3.

The evaluation of the above indicators was performed at the same spot as that where the GR expression was evaluated, and the correlation between them was analyzed. When the variable of every indicator was compered between Pattern (0, 1) group and Pattern (2, 3) group of GR expression, the expansion of the MUC5AC-positive scattered cell area thickness beneath the pit (P <0.01) and that of MUC6-psotive cell area thickness (P=0.0229) were significantly correlated with Pattern (2, 3) group of GR expression (Table 2, Fig. 4 and Fig. 5). Here, regardless of the GR-distribution pattern, the ratio of the MUC5AC-positive scattered cell area thickness beneath the pit to total thickness of the mucosa showed significant correlation with the ratio of the MUC6-psotive cell area thickness to the total thickness of the mucosa based on the Pearson’s correlation analysis (P <0.01).

We also conducted analyses on the correlation between the above indicators

and the PPI administration status of the patients, also resulting in that the tendency of the expansion of the MUC5AC-positive scattered cell area thickness beneath the pit (P <0.01) and that of MUC6-psotive cell area thickness (P <0.01) were significantly correlated with the administration history of PPI, with the tendency of the thicker mucosa and gland area in the PPI cases compared with the non-PPI cases (Table 2, Fig. 4 and Fig. 5).

3.5. GR expression in fundic gland polyps

In the FGPs examined here, the IHC of GR showed intense and diffuse membranous linear positivity in almost all of the cases (Fig. 6C). The intensity scores (see Supplementary of the results for their definition) were evaluated as follows (shown in the order of Score 0/1/2/3): 0/0/1/42 in the upper half of the oxyntic gland area and 0/1/6/36 in the lower half of the oxyntic gland area, and no distribution deviation of GR expression was observed in the polyps.

Analyzing the correlation between the demographic/clinical variables or histologic features of the FGP cases and the PPI administration status of the patients, the size of the polyp was significantly larger (P <0.01), and foveolar cell hyperplasia-like change and parietal cell change were significantly more frequently observed in the PPI cases compared with the non-PPI cases (P <0.01) (Table 3, Fig. 6A and 6B).

4. Discussion

In the present study, the expression of GR seemed to be observed in every parietal cell in both protein and mRNA levels. However, the

immunohistochemistry revealed unique membranous linear expression of GR as well as cytoplasmic expression, and the parietal cells with the former type of GR expression were limitedly distributed in oxyntic gland mucosa. A previous study had reported similar membranous and cytoplastic expression of GR in parietal cells [3], which, however, had not been concerned with their distribution in the mucosa. Here, we investigated the expression of GR in gastric oxyntic gland mucosa focusing on the distribution of the unique membranous linear expression.

The present results revealed that the parietal cells with GR membranous linear expression were limited just around the isthmus-neck region in some cases, but were also distributed in the gland base of oxyntic glands in other cases. These distribution patterns were significantly correlated with the status of component cell compartmentalization in oxyntic gland mucosa, that is, the membranous linear expression of GR in deep oxyntic glands was more frequently observed in the cases which showed the expansion of the MUC5AC-positive scatted cell area beneath the pit and that of the MUC6-positive cell area. In the human oxyntic gland mucosa, progenitor cells in the isthmus region proliferate and differentiate into mature cells as they migrate bidirectionally, upward to the pit and downward to the gland base [21,22]. In normal conditions of this cell renewal system, MUC5AC-positive surface mucous cells are mostly confined to the pit whereas MUC6-positive mucous neck cell are identified limitedly in the neck region [21,23,24]. However, these normal cell organization and compartmentalization would derange in some mucosal damage. MUC6-positive cells in deep glands are frequently observed as pseudopyloric metaplasia or pyloric metaplasia (or also referred to as spasmolytic polypeptide-expressing metaplasia: SPEM) in the

recovery process from gastritis or ulcer, or in the background mucosa next to gastric carcinoma [25-27]. Thus, the present results seem to suggest that the membranous linear GR expression in parietal cells could be related to maintaining the component cell organization, and its distribution change could also reflect the derangement of the organization and compartmentalization.

The involvement of the Gastrin-GR system in maintaining mucosal organization has been suggested in previous experimental studies. Gastrin or GR deficient mice showed a decreased number of parietal cells and ECL cells, and an increased number of H^+-K^+-ATPase-negative immature parietal cells [28,29]. In addition, severe atrophy of the gastric mucosa due to a decreased number of parietal cells and ECL cells was observed even in hypergastrinemia [30]. With regard to the parietal cells, in the mice or rats model which caused apoptosis to parietal cells selectively, a decreased number of chief cells and endocrine cells was observed in addition to hyperplasia of superficial mucous epithelial cells and an appearance of SPEM-like mucous cells in oxyntic glands [31-33].

Another important point of the present results was that the history of PPI use seemed to be related both to the membranous linear GR expression of parietal cells in deep oxyntic glands and, to the expansion of the MUC5AC-positive scatted cell area beneath the pit and that of the MUC6-positive cell area, which suggests that PPIs could cause the derangement of gastric mucosal organization with the GR distribution change. However, the GR distribution alteration was not related to foveolar cell hyperplasia-like change and parietal cell change, which are typical gastric mucosal changes related to PPI use [14,15]. Therefore, the derangement of gastric mucosal organization with the GR distribution change

observed in the present study could be independent of the histologic changes so far reported, and more profound alteration which should be noticed with regard to the PPI medication.

FGPs are unique polypoid lesions characterized by distorted architecture of oxyntic glands (tortuous glands with multiple buds and cystic lumens often lined by foveolar type mucous cells), and aberrant appearance of proliferating cells in the gland area [34]. They arise sporadically, but have also been known to arise and enlarge in relation to the long-term use of PPIs with the characteristic histologic changes including larger cystic lumens with foveolar type cells, foveolar cell hyperplasia, parietal cell protrusion, and more proliferating cells in the deep gland area [14,15,35], some of which were confirmed in the present study. Besides, the present study revealed that the GR membranous linear expression of parietal cells was intense and diffusely distributed in the most FGPs examined, suggesting that the GR expression pattern could be related to the deranged gland organization characterizing FGPs as well as the findings in oxyntic gland mucosa. However, the GR expression pattern was the same regardless of the medication history of PPI use. Thus, even if PPIs are a contributing factor in the development and enlargement of FGPs, it seems to be necessary to clarify their more fundamental basis.

5. Conclusions

The present study revealed the unique GR membranous linear expression in parietal cells. Its distribution mode in oxyntic gland mucosa and FGPs could be related to deranged gland architecture, suggesting that the GR expression would

play a role in maintaining the mucosal organization, the mechanism of which is yet to be uncovered however. The limitations of the study are a relatively small sample size, arbitrary sample selection, and some insufficiency of information of patients such as status of *Helicobacter pylori* infection.

Appendix A. Supplementary data

Authors' contributions:

Y.S. and S.B. contributed to the research design. Y.S., Y.K. and T.M. contributed to data acquisition. Y.S. and S.B. contributed data analysis and interpretation. Y.S. wrote the draft of the manuscript, and all authors reviewed and edited the manuscript.

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Figure legends:

Fig. 1. The mode of GR expression in parietal cells of oxyntic gland mucosa on IHC, IF and ISH. A, The HE preparation of the isthmus-neck region of oxyntic gland mucosa shows a number of parietal cells with central rounded nuclei and finely granular eosinophilic cytoplasm. B, The IHC of GR protein shows membranous linear positivity as well as weak cytoplasmic staining of the cells morphologically consistent with the parietal cells in A. C, The IF for the detection of GR protein shows the positivity similar to B, that is, membranous linear fluorescence as well as weak cytoplasmic fluorescence without nuclear staining. D, The ISH for the detection of GR mRNA shows dot-like signals in the parietal cells. Measure bar = 50 μm in A and B, 100 μm in C, and 20 μm in D.

Fig. 2. The IHC findings of oxyntic gland mucosa representing GR distribution patterns (0 to 3) with regard to parietal cells. A, Pattern 0: only cytoplasmic expression. B, Pattern 1: membranous linear expression just around the isthmus-neck region (sandwiched by arrows). C, Pattern 2: membranous linear expression both in the isthmus-neck region and in the base region of fundic glands dividedly (sandwiched by arrows). D, Pattern 3: membranous linear expression from the isthmus-neck to the base region continuously. Measure bar = 100 μm in A to D.

Fig. 3. Histological and IHC findings as indicators to evaluate the status of component cell compartmentalization in oxyntic gland mucosa. A, Schematic illustration of one tubular invagination of intact oxyntic gland mucosa, consisting of pit, isthmus, neck, and base from the superficial epithelium to the bottom of the

gland accordingly. B, The HE preparation of oxyntic gland mucosa depicting the measurement of (a) total thickness of the mucosa and (b) thickness of the glands including the isthmus and neck. C, MUC5AC IHC depicting the measurement of (c) thickness of the MUC5AC-positive pit (surface mucous cells), (d) thickness of the MUC5AC-positive scattered cell area beneath the pit, and (e) thickness of (c) + (d). D, MUC6 IHC depicting the measurement of (f) thickness of MUC6-positive cell area. E, Ki-67 IHC depicting the measurement of (g) thickness of Ki-67-positive proliferating zone. The Ki-i67 positive rate (%) of the gland area except for the proliferating zone (h) is also evaluated. Measure bar = 200 μm.

Fig. 4. The IHC findings of oxyntic gland mucosa in a representative case with PPI medication. A, HE preparation showing a full thickness of oxyntic gland mucosa from the pit to the base with mild parietal cell protrusion, which is presumably related to the PPI medication. B, MUC5AC IHC showing irregularly scattered positive cells beneath the pit, some reaching the near gland base, in addition to the positivity of the pit foveolar cells. C, MUC6 IHC showing the expanded positive cell area in the gland, partly reaching the near gland base. D-F, GR IHC showing the full thickness of the mucosa (D), the pit to around neck region (E) corresponding to the upper blue rectangle area in D, and the around base region (F) corresponding to the lower blue rectangle area in D. The membranous linear positivity of parietal cells is observed from the isthmus/neck to the gland base (D), being categorized to Pattern 3 of the GR distribution classification, with the GR intensity of Score 3 in E and Score 2 in F, respectively. Measure bar = 200 μm in A to D, and 100 μm in E and F.

Fig. 5. The IHC findings of oxyntic gland mucosa in a representative case without PPI medication. A, HE preparation showing a full thickness of oxyntic gland mucosa from the pit to the base with unremarkable change. B, MUC5AC IHC showing the positivity of the pit foveolar cells with a few scattered positive cells beneath the pit. C, MUC6 IHC showing the limited thin layer of positive cells beneath the pit. D-F, GR IHC showing the full thickness of the mucosa (D), the pit to around neck region (E) corresponding to the upper blue rectangle area in D, and the around base region (F) corresponding to the lower blue rectangle area in D. Intense membranous linear positivity of parietal cells (Score 3 of the GR intensity) is limited around the neck region (D, E), and no membranous linear positivity is observed around the base of the gland (Score 0 of the GR intensity, D, F), being categorized to Pattern 1 of of the GR distribution classification. Measure bar = 200 μm in A to D, and 100 μm in E and F.

Fig. 6. Histological and GR IHC findings in a representative case of FGP obtained from a patient with PPI medication. A, HE preparation showing cyst-like irregularly dilated oxyntic glands with parietal cell protrusion. B, Superficial foveolar epithelium and foveolar-like epithelium in a dilated gland of the deep portion of FGP, both representing the cytoplasm rich in mucous and hyperplastic-like change (arrows). A moderate degree of mononuclear cell infiltration is observed in the lamina propria. C, GR IHC showing diffuse intense membranous linear positivity (Score 3 of GR intensity) of parietal cells in the dilated oxyntic glands. Measure bar = 100 μm in A and B, and 50 μm in C.