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European Journal of Pharmacology 502 (2004) 243–252

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Unique roles of G protein-coupled histamine H_2 and gastrin receptors in growth and differentiation of gastric mucosa

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Received 10 June 2004; received in revised form 20 August 2004; accepted 1 September 2004
Available online 1 October 2004

Abstract

Disruption of histamine H_2 receptor and gastrin receptor had different effects growth of gastric mucosa: hypertrophy and atrophy, respectively. To clarify the roles of gastrin and histamine H_2 receptors in gastric mucosa, mice deficient in both (double-null mice) were generated and analyzed. Double-null mice exhibited atrophy of gastric mucosae, marked hypergastrinemia and higher gastric pH than gastrin receptor-null mice, which were unresponsive even to carbachol. Comparison of gastric mucosae from 10-week-old wild-type, histamine H_2 receptor-null, gastrin receptor-null and double-null mice revealed unique roles of these receptors in gastric mucosal homeostasis. While small parietal cells and increases in the number and mucin contents of mucous neck cells were secondary to impaired acid production, the histamine H_2 receptor was responsible for chief cell maturation in terms of pepsinogen expression and type III mucin. In double-null and gastrin receptor-null mice, despite gastric mucosal atrophy, surface mucous cells were significantly increased, in contrast to gastrin-null mice. Thus, it is conceivable that gastrin-gene product(s) other than gastrin-17, in the stimulated state, may exert proliferative actions on surface mucous cells independently of the histamine H_2 receptor. These findings provide evidence that different G-protein coupled-receptors affect differentiation into different cell lineages derived from common stem cells in gastric mucosa.

Keywords: G protein; Histamine H_2; Double-null, mouse

1. Introduction

Recently, gene-targeting techniques have made it possible to generate mice deficient in a number of genes involved in gastric acid secretion (Friis-Hansen et al., 1998; Fukushima et al., 2003; Kobayashi et al., 2000; Koh et al., 1997; Langhans et al., 1997; Lloyd et al., 1997; Matsui et al., 2000; Nagata et al., 1996; Tanaka et al., 2002). Of these gene products, histamine H_2, gastrin, and muscarine M_3 receptors are direct targets of secretagogues and are involved in acid production in parietal cells. Targeted disruption of the histamine H_2 receptor caused hypertrophy of gastric mucosa due to marked hyperplasia of parietal, mucous neck and enterochromaffin-like (ECL) cells (Fukushima et al., 2003). Despite prominent hypergastrinemia, surface mucous cells were not as increased in number as downward migrating cells in histamine H_2 receptor-null mice (Fukushima et al., 2003). In contrast, gastrin receptor-null mice exhibited remarkable

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doi:10.1016/j.ejphar.2004.09.013

gastric mucosae atrophy accompanied by decreases in parietal and ECL cell numbers (Nagata et al., 1996). Although differences in pH values between wild-type mice and histamine H_2 receptor-null mice were minimal (Fukushima et al., 2003; Kobayashi et al., 2000), gastrin-dependent acid production was impaired in histamine H_2 receptor-null mice. In gastrin receptor-null mice, basal acid productions were lower than those in wild-type mice (Langhans et al., 1997; Nagata et al., 1996). In this study, to further clarify the distinct roles of histamine H_2 receptor and gastrin receptor in gastric mucosa, mice deficient in both the histamine H_2 and the gastrin receptors (double-null mice) were generated. We also analyzed gastric mucosa from aged histamine H_2 receptor-null mice and aged double-null mice. Herein, we present evidence that these different G-protein coupled-receptors mediate differentiation into different cell lineages derived from common stem cells in gastric mucosa.

2. Materials and methods

2.1. Mice

All animal experimental procedures were reviewed and approved by the Institutional Animal Care and Research Advisory Committee of the University of Tokyo. Mice deficient in histamine H_2 receptors were generated as described previously (Fukushima et al., 2003; Shindo et al., 2002).

2.2. Generation of mice deficient in both the histamine H_2 receptor and the gastrin receptor (double-null mice)

Histamine H_2 receptor-null mice and gastrin receptor-null mice with the genetic background of the 129/Sv×C57BL/6 hybrid were used (Fukushima et al., 2003; Nagata et al., 1996). Offspring obtained by crossing histamine H_2 receptor-null and gastrin receptor-null mice were confirmed to be heterozygous for both the histamine H_2 receptor and the gastrin receptor. These mice were then crossed and the offspring thus obtained were genotyped with PCR and/or Southern blot analysis using genomic DNA prepared from tail biopsies. Of these offspring, wild-type, histamine H_2 receptor-null, gastrin receptor-null and double-null mice were used for the following studies. Double-null mice appeared normal, were healthy into adulthood and both sexes were fertile.

2.3. Generation of polyclonal antibody against murine pepsinogen C

Polyclonal antibody against murine pepsinogen C was generated by a previously described method (Fukushima et al., 1993). The 100 carboxyl-terminal amino acids of murine pepsinogen C were fused to Glutathione *S*-transferase, which was used to immunize female New Zealand white rabbits. Serum collected from the immunized rabbits was passed through Affigel-10 beads, which had been cross-linked to Glutathione *S*-transferase. The flow-through was collected and passed through Afvfigel-10 beads, which had been cross-linked to the fusion protein. Antibody adsorbed to the beads was collected. This polyclonal antibody specifically recognizes chief cells in mouse oxyntic mucosa.

2.4. Histological analysis

Gastric specimens were fixed in 3% phosphate-buffered paraformaldehyde (pH 7.4), embedded in paraffin, and cut into 3 μm sections. The sections were stained with periodic acid-Schiff (PAS), hematoxylin and eosin, and examined under a light microscope. Paraffin-embedded gastric tissue sections were dewaxed and rehydrated with graded concentrations of ethanol. After treatment with 2% H_2O_2/phosphate buffered saline for 10 min, tissue sections were incubated with anti-pepsinogen C antibody, anti-histidine decarboxylase (HDC) polyclonal antibody, anti-$H^{(+)}/K^{(+)}$-ATPase monoclonal antibody (Fukushima et al., 1999), anti-type III mucin monoclonal antibody HIK1087 (Kanto-Kagaku, Japan) or normal rabbit or mouse immunoglobulin G (IgG) overnight at 4 °C. The sections were rinsed and then incubated for 30 min with biotinylated anti-rabbit or mouse IgG (1:400 dilution). The tissue sections were then rinsed and incubated for 30 min with peroxidase-labeled streptavidin (1:70 dilution). The slides were rinsed again in phosphate buffered saline and reacted with diaminobenzidine for 5 min at room temperature. Finally, the sections were rinsed and counterstained with hematoxylin.

2.5. Incorporation of the thymidine analog bromodeoxyuridine (BrdU)

BrdU (80 mg/kg BW(body weight)) was injected intraperitoneally into mice 2 h before sacrifice. Gastric tissues were removed and fixed in 3% phosphate-buffered paraformaldehyde. Immunohistochemistry with anti-BrdU monoclonal antibody was performed using paraffin-embedded sections from these samples.

2.6. Measurement of gastric pH

Wild-type and histamine H_2 receptor-null mice were fasted overnight with free access to water. At 1.5 h after subcutaneous injection of vehicle (0.5% methylcellulose), 10 mg/kg BW of famotidine, 10 mg/kg BW of pirenzepine dihydrochloride (a muscarine M_1 receptor antagonist) or 10 mg/kg BW of (*R*)-1-[2,3-dihydro-1-(2′ -methylphenacyl)-2-oxo-5-phenyl-1H-1,4-benzodiazepin-3-yl]-3-(3-methylphenyl)urea (YM022), a gastrin receptor antagonist, the mice were sacrificed and their stomachs were immediately excised. Gastric pH was measured using an ultra-thin pH monitor (Horiba, Japan).

2.7. Measurement of secretagogue-induced acid secretion

Mice were maintained on anesthesia in chambers infused with oxygen gas saturated with diethylether. The stomach and duodenum were exposed via an epigastric midline incision. A tube inserted from the duodenum was placed in the gastric lumen. Stomachs were washed with 1 ml of prewarmed physiologic saline three times. After extraction of the tube and ligation of the pylorus, physiologic saline or secretagogue solution was administered peritoneally. A total of 10 mg/kg BW of histamine dihydrochloride, 0.05 mg/kg BW of carbachol or 0.1 mg/kg BW of gastrin-17 were administered, i.e. 2.5 ml/kg BW of physiologic saline as a control, histamine dihydrochloride solution (4 mg/ml), carbachol solution (0.02 mg/ml) or gastrin-17 solution (0.04 mg/ml). Thirty minutes after administration, the mice were sacrificed and their stomachs were excised. Gastric juice was collected with 1.5 ml of physiologic saline. Secreted gastric acid was measured by titrating the collected gastric juice to pH 7.0.

2.8. Statistical analysis

Quantitative values were expressed as means±S.E. Statistical significance was tested using the unpaired *t*-test (two tailed). A value of $P<0.05$ was considered significant.

3. Results

3.1. Comparison of gastric mucosae and serum gastrin levels of 10-week-old histamine H_2 receptor-null, gastrin receptor-null, double-null and wild-type mice

Stomachs from 10-week-old double-null mice weighed significantly less than those of 10-week-old wild-type mice

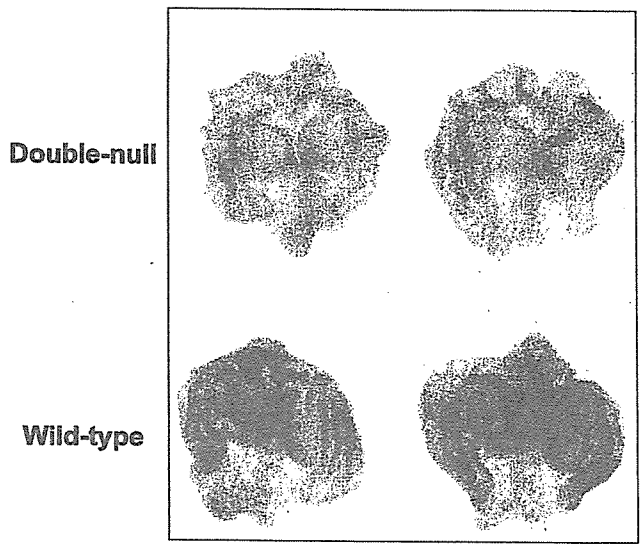

Fig. 1. Macroscopic views of stomachs from 10-week-old wild-type and double-null mice. The excised stomachs were opened along the greater curvature.

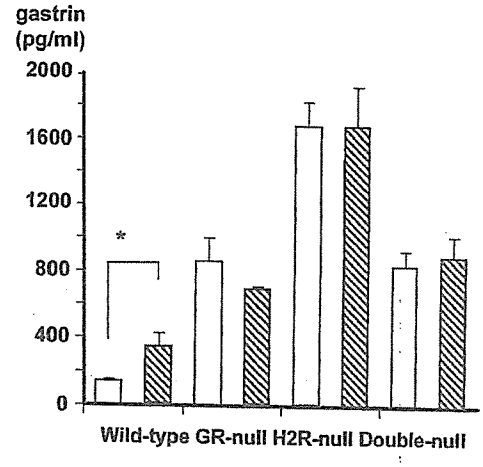

Fig. 2. Serum gastrin levels in wild-type, histamine H_2 receptor-null, gastrin receptor-null and double-null mice. Serum gastrin levels were measured in fasting (open bars) and fed states (hatched bars) in 10- to 12-week-old wild-type, histamine H_2 receptor-null (H2R-null), gastrin receptor-null (GR-null) and double-null mice. Data are presented as means±S.E. (n=15). *$P<0.0001$ between fasting and fed states.

(double-null 60.0±0.6 g/kg BW, wild-type 79.0±1.0 g/kg BW, $P<0.0001$). Macroscopically, oxyntic mucosae from double-null mice were more atrophic than those from wild-type mice (Fig. 1). Serum gastrin levels in double-null mice were significantly higher than those in wild-type mice, while being comparable to and lower than those in gastrin receptor-null mice and histamine H_2 receptor-null mice, respectively (Fig. 2). In addition, except in wild-type mice serum gastrin levels were not elevated by feeding (Fig. 2).

To explore the effects of disrupting gastrin receptor and histamine H_2 receptor genes, we examined oxyntic mucosae from the four types of mice at 10 weeks of age, PAS staining of gastric mucosa from 10-week-old double-null mice showed no hypertrophy of oxyntic mucosae in double-null mice (Fig. 3D).

In histamine H_2 receptor-null mice, oxyntic mucosal hypertrophy was attributable to hyperplasia of ECL, parietal and mucous neck cells, and parietal cells were small (Table 1). In some portions of oxyntic mucosae from histamine H_2 receptor-null mice, peculiar mucous neck cells full of mucin protruded into the gastric gland lumen. Despite marked hypergastrinemia surface mucous cells were not as increased in number as the downward migrating cells, resulting in a decreased percentage of surface mucous cells per gland in histamine H_2 receptor-null mice. These findings confirm our previous report on histamine H_2 receptor-null mice (Table 1, Fig. 3B) (Fukushima et al., 2003). However, on closer examination, we found the number of surface mucous cells to be significantly increased as compared to wild-type mice (Table 1).

In gastrin receptor-null mice, numbers of downward migrating cells were decreased as previously reported ($P<0.001$, vs. wild-type mice) (Table 1) (Nagata et al., 1996). Interestingly, surface mucous cell cells were increased in number as compared with wild-type mice (26.7±1.6

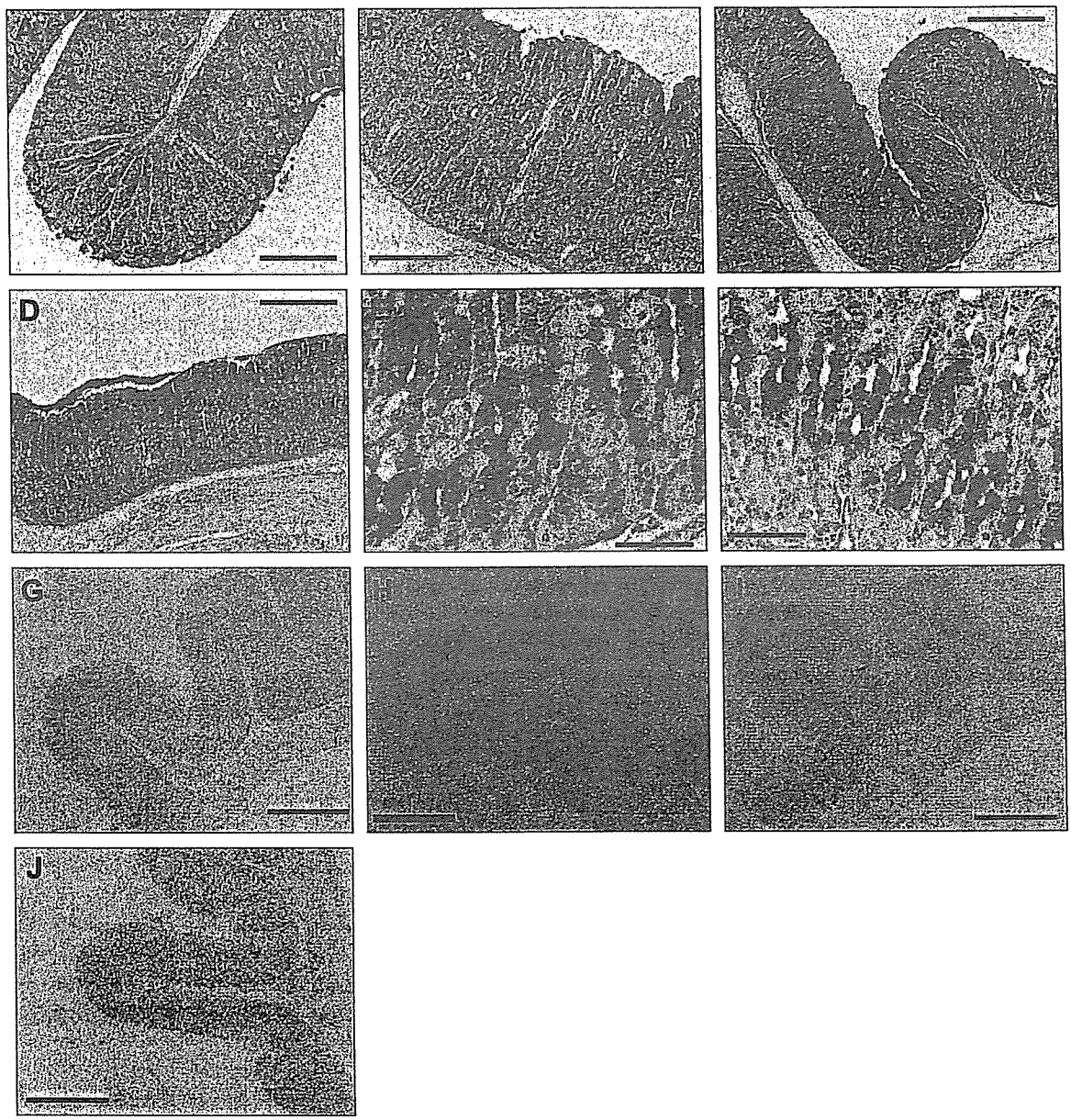

Fig. 3. Oxyntic mucosa from 10-week-old wild-type, histamine H_2 receptor-null, gastrin receptor-null and double-null mice. Sections of oxyntic mucosa from wild-type (A, G), histamine H_2 receptor-null (B, H), gastrin receptor-null (C, E, I) and double-null (D, F, J) mice were subjected to PAS staining (A, B, C, D, E, F) or BrdU labeling (G, H, I, J). Scale bars, 200 μm (A, B, C, D), 50 μm (E, F), 500 μm (G, H, I, J).

arbitrary units per gland vs. 20.3±0.5 arbitrary units per gland, $P<0.001$) (Table 1, Fig. 3C). Thus, although numbers of downward migrating cells were decreased, the total number of cells per gland did not differ significantly between gastrin receptor-null and wild-type mice (Table 1). In addition, an increase in the number of BrdU positive cells per gland was observed in gastrin receptor-null mice (gastrin receptor-null, 2.76±0.14 arbitrary units per gland, wild-type, 0.95±0.09 arbitrary units per gland, $P<0.001$) (Table 1, Fig. 3I). Just as in histamine H_2 receptor-null mice, some portions of the oxyntic mucosa, especially at the greater curvature and near the antrum, contained mucous neck cells full of mucins (Fig. 3E). Small parietal cells were observed in gastrin receptor-null mice as well (gastrin receptor-null mice, 5.37±0.10 arbitrary units per cell, wild-type mice, 8.86±0.17 arbitrary units per cell, $P<0.001$) (Table 1). In double-null mice, numbers of ECL cells, and parietal cells as well as the total number of downward migrating cells, were decreased (Table 1). As in gastrin receptor-null mice, the number of surface mucous cells was increased as compared with those from wild-type mice (25.3±0.8 arbitrary units per gland vs. 20.3±0.5 arbitrary units per gland, $P<0.001$) (Table 1, Fig. 3D). BrdU positive cells per gland were increased in number in double-null mice (double-null, 1.75±0.13 arbitrary units per gland, wild-type, 0.95±0.09 arbitrary units per gland, $P<0.001$) (Table 1, Fig. 3J). Total number of cells per gland did not differ significantly between wild-type and double-null mice (Table 1). Mucous neck cells with

Table 1
Quantitative analyses of gastric glands from 10-week-old wild-type, histamine H_2 receptor-null, gastrin receptor-null and double-null mice

	Total cell number	Surface mucous cell number	Gland cell number	Parietal cell		ECL cell number	BrdU positive cell number
				Number	Size		
Wild-type	63.7±0.7	20.3±0.5	43.4±0.7	20.3±0.4	8.86±0.17	1.44±0.13	0.95±0.09
Histamine H_2 receptor-null	114.2±3.6[a]	26.7±1.6[a]	87.5±2.9[a]	38.6±1.3[a]	4.79±0.11[a]	7.61±0.32[a]	2.01±0.11[a]
Gastrin receptor-null	61.5±1.2	26.7±0.7[a]	34.8±0.9[a]	13.8±0.2[a]	5.37±0.10[a]	0.53±0.08[a]	2.76±0.14[a]
Double-null	61.1±1.1	25.3±0.8[a]	35.8±0.8[a]	14.2±0.4[a]	5.01±0.09[a]	0.81± 0.07[a]	1.75±0.13[a]

Numbers of cells were counted in gastric glands sectioned centrally and in a manner parallel to their longitudinal axes, then expressed as arbitrary units per gland. Parietal cell size was determined by measuring the longitudinal cross sectional area of parietal cells from these gastric glands and expressed as arbitrary units per cell. One hundred glands from 10 mice (10 glands per mouse) were used for each type of mouse. Data are expressed as arbitrary units per gland or parietal cell since the data obtained are proportional but not equivalent to the actual cell numbers or parietal cell mass.

[a] $P<0.0001$ vs. wild-type mice.

characteristics similar to those in histamine H_2 receptor-null mice and gastrin receptor-null mice were seen in similar portions of the gastric mucosa (Fig. 3F). Small parietal cells were also observed in double-null mice (double-null mice, 5.01±0.09 arbitrary units per cell, wild-type mice, 8.86±0.17 arbitrary units per cell, $P<0.001$) (Table 1).

3.2. Comparison of chief cell lineage in gastric mucosae from 10-week-old wild-type, histamine H_2 receptor-null, gastrin receptor-null and double-null mice

Next, to explore the effects of histamine H_2 receptor and gastrin receptors on maturation of the chief cell lineage, expressions of pepsinogen and type III mucin were examined in gastric glands in each type of mouse. Fig. 4 is a schematic representation of a gastric gland. Fig. 5 shows that type III mucin positive cells were increased in number in histamine H_2 receptor-null, gastrin receptor-null and double-null mice as compared with wild-type mice. In addition, type III mucin positive cells, although present in the base regions of gastric glands from histamine H_2 receptor and double-null mice (Fig. 5J,L), were very scarce at the bases of gastric glands from wild-type and gastrin receptor-null mice (Fig. 5I,K). In wild-type mice, numbers of pepsinogen positive cells in gastric glands gradually increased from the isthmus to the base and pepsinogen expression per cell had already peaked in the neck region (Fig. 5A). In gastrin receptor-null mice, pepsinogen expression in gastric glands was maximal only at the base (Fig. 5C). It is noteworthy that mature chief cells, without type III mucin and with abundant pepsinogen, were present at the base region of gastric glands from gastrin receptor-null mice (Fig. 5C,G). In contrast, gland cells with abundant pepsinogen expression and without type III mucin were not present in histamine H_2 receptor-null mice and double-null mice (Fig. 5B,D,F,H). In addition to the low pepsinogen expression, pepsinogen levels per cell did not increase from the isthmus to the base in histamine H_2 receptor-null and double-null mice (Fig. 5B,D).

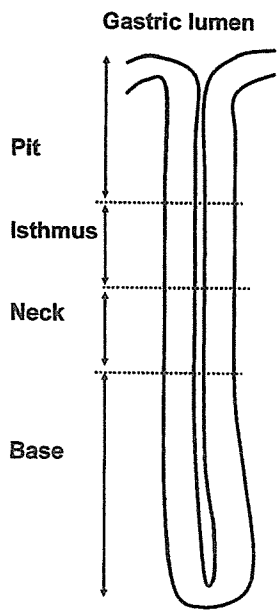

Fig. 4. Schematic drawing of a gastric gland.

3.3. Gastric pH and gastric acid productions in 10-week-old wild-type, histamine H_2 receptor-null, gastrin receptor-null and double-null mice

First, in vivo acid productions in response to secretagogues were measured. Histamine H_2 receptor-null mice were responsive to carbachol, but not to histamine or gastrin-17 (Fukushima et al., 2003). Secretagogue-induced acid secretion (10 mg/kg BW of histamine, 0.05 mg/kg BW of carbachol) was not observed in either gastrin receptor-null nor double-null mice (data not shown). Gastric pH values in double-null mice were the highest among the four types of mice (Fig. 6). Those in gastrin receptor-null mice were higher than those in wild-type or histamine H_2 receptor-null mice and lower than those in double-null mice. Treatment of gastrin receptor-null mice with famotidine (10 mg/kg BW) or pirenzepine (10 mg/kg BW) raised gastric pH values, indicating that histaminergic and muscarine pathways, although severely impaired, are functional in gastrin receptor-null mice. Because fasting gastric pH values in double-null mice were too high to assess the inhibitory effects of pirenzepine, the effect of

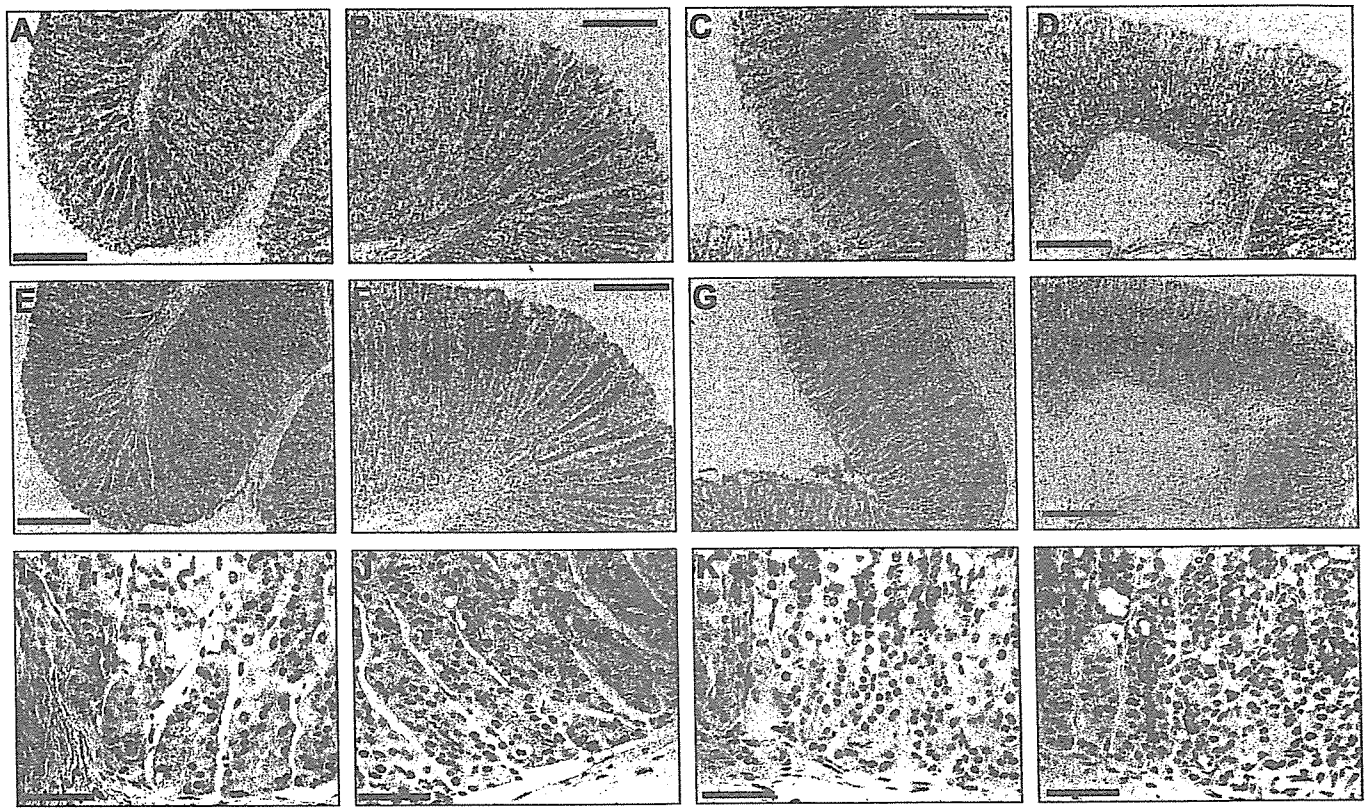

Fig. 5. Expressions of pepsinogen and type III mucin in oxyntic mucosa from 10-week-old wild-type, histamine H_2 receptor-null, gastrin receptor-null and double-null mice. Sections of oxyntic mucosa from wild-type (A, E, I), histamine H_2 receptor-null (B, F, J), gastrin receptor-null (C, G, K) and double-null (D, H, L) mice were stained with anti-pepsinogen antibody (A, B, C, D) and anti-type III mucin antibody (E, F, G, H, I, J, K, L). In I, J, K, L, type III mucin-positive cells are marked with asterisks. Scale bars, 200 μm (A, B, C, D, E, F, G, H), 50 μm (I, J, K, L).

carbachol at 1 mg/kg BW, a dose which is too high to be tolerated in measuring in vivo acid production, was examined in double-null mice. Fig. 6 shows that while gastrin receptor-null mice were responsive to both histamine and carbachol, double-null mice were unresponsive to both.

3.4. Long term follow-up of histamine H_2 receptor-null mice and double-null mice

At 6 months, while there were no changes in gastric mucosa from wild-type mice, further elongation of gastric glands was observed in histamine H_2 receptor-null mice

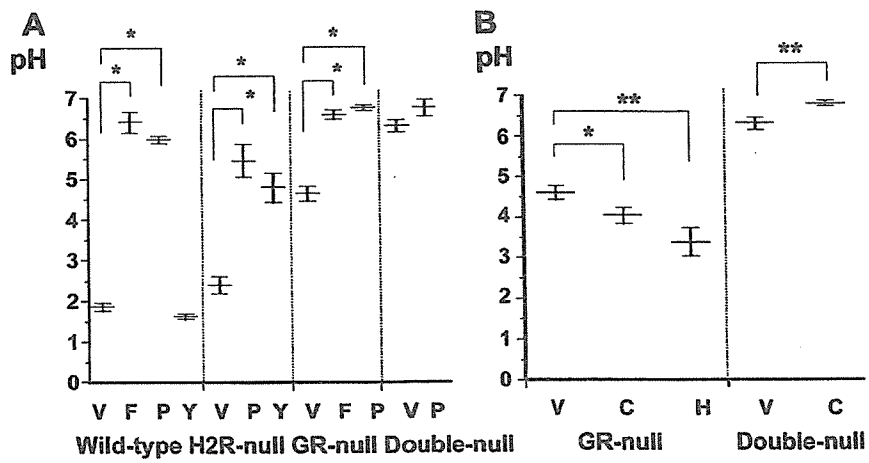

Fig. 6. Gastric pH in wild-type, histamine H2 receptor-null, gastrin receptor-null and double-null mice. Wild-type, histamine H_2 receptor-null (H2R-null), gastrin receptor-null (GR-null) and double-null (10 to 12 weeks old) mice were fasted overnight with free access to water. (A) At 1.5 h after subcutaneous injection of 0.5% methylcellulose as a vehicle (V) (n=20), 10 mg/kg BW of famotidine (F) (n=20), 10 mg/kg BW of pirenzepine (P) (n=20) or 10 mg/kg BW of YM022 (Y) (n=20), the mice were killed and their stomachs immediately excised. Gastric pH was measured using an ultra-thin pH monitor. (B) At 15 min after subcutaneous injection of vehicle (V) (n=20), 10 mg/kg BW of histamine (H) (n=20) or 1 mg/kg BW of carbachol (C) (n=20), the mice were killed and their stomachs immediately excised. Gastric pH was measured using an ultra-thin pH monitor. Data are presented as means±S.E. *P<0.001 vs. respective values.

Table 2
Stomach weight and gastric pH in aged wild-type and aged histamine H_2 receptor-null mice

	Stomach weight (g)	Fasting gastric pH
Wild-type	0.16±0.02	1.61±0.13
Histamine H_2 receptor-null	0.38±0.02[a]	2.14±0.13[b]

Stomach weight and fasting gastric pH were measured in 12-month-old wild-type and histamine H_2 receptor-null mice. Data are expressed as means±S.E. (n=10, each group).
[a] P<0.0001 vs. wild-type mice.
[b] P=0.0134 vs. wild-type mice.

(data not shown). However, the structure of gastric oxyntic mucosa from 6-month-old histamine H_2 receptor-null mice was very similar to that of mucosa from 10-week-old histamine H_2 receptor-null mice, except for the presence of cysts near the basal region. In 12-month-old histamine H_2 receptor-null mice, in addition to the marked increase in stomach weight (Table 2), oxyntic mucosal structures appeared to differ strikingly from those of wild-type and younger histamine H_2 receptor-null mice (Fig. 7B). Oxyntic mucosa from aged histamine H_2 receptor-null mice was full of cystic structures (Fig. 7B). Most gastric glands were dilated and, in addition, interstitial tissues between cysts were markedly increased (Fig. 7D), which is in sharp contrast to the findings in gastric mucosa from aged wild-type mice (Fig. 7C). Some cells lining the cysts were positive for $H^{(+)}/K^{(+)}$-ATPase, pepsinogen and HDC (Fig. 7E,F,G), indicating that the cysts were derived from dilated gastric glands. However, small portions of oxyntic mucosa remained mostly unaltered (Fig. 7H), suggesting that the program for formation of normal gastric glands is preserved in gastric mucosal stem cells. Gastric pH values in aged histamine H_2 receptor-null mice were essentially preserved (Table 2). Similar features were observed in gastric mucosae from 24-month-old histamine H_2 receptor-null mice (data not shown). Unlike histamine H_2 receptor-null mice, there were no significant differences in oxyntic mucosae between 10-week-old and 12-month-old double null mice (data not shown).

4. Discussion

Oxyntic mucosal atrophy in double-null mice confirms the oxyntic mucosal hypertrophy observed in histamine H_2 receptor-null mice to be due to stimuli delivered via gastrin receptors. In double-null and gastrin receptor-null mice, numbers of gland cells as a whole (downward migrating cells) were decreased. However, despite gastric mucosal atrophy surface mucous cell number was moderately but significantly increased in gastrin receptor-null and double-null mice as compared with wild-type mice (Table 1). Turnover of surface mucous cells is far faster than that of downward migrating cells (Karam and Leblond, 1992, 1993a,b,c,d, 1995). Thus, it is likely that most of the increases in BrdU labeling in oxyntic mucosae in gastrin receptor-null and double-null mice are attributable to increased growth and differentiation into surface mucous cells. In the case of gastrin-null mice, the percentage of BrdU positive cells in oxyntic mucosa was not different from that in wild-type mice and there was a marked decrease in the surface mucous cells in gastrin-null mice as compared with wild-type mice (Koh et al., 1997). Thus, gastric mucosae from gastrin receptor-null and double-null mice and those from gastrin-null mice are different in terms of number of surface mucous cells. Post-translational modification of preprogastrin yields progastrin and glycine-extended gastrin as well as gastrin-17 (Dockray et al., 2001). In G-cells, gastric mucosal processing of preprogastrin yields gastrin and glycine-extended gastrin (Dockray et al., 2001). Glycine-extended gastrin reportedly has very low affinity for the gastrin receptor and has been suggested to interact with a novel receptor, which remains to be identified (Dockray et al., 2001). Thus, serum and oxyntic mucosal levels of glycine-extended gastrin may well be elevated, like those of gastrin-17, in gastrin receptor-null and double-null mice. In a study using gastrin-null mice, infusion of gastrin-17 and glycine-extended gastrin had distinct effects on gastric acid secretion, via different signal transduction pathways (Chen et al., 2000; Hollande et al., 2001; Stepan et al., 1999). Thus, the absence of glycine-extended gastrin effects in gastrin-null mice and possible hyperstimulation of the glycine-extended gastrin receptor in gastrin receptor-null mice might account for the difference in surface mucus cells in these mice. The finding of similar surface mucous cell increases in double-null mice indicates that a glycine-extended gastrin-dependent increase in surface mucous cells in the absence of gastrin receptors is not dependent on the histamine H_2 receptor. We speculate that a similar increase in surface mucous cell number in histamine H_2 receptor-null mice was caused by such a glycine-extended gastrin effect. Taken together, our results show gastrin and glycine extended-gastrin to have distinct roles in the growth of gastric mucosa.

We previously reported that maturation of the chief cell lineage was impaired in gastric mucosa from histamine H_2 receptor-null mice (Fukushima et al., 2003). In this report, mature chief cells, which we define as being positive for pepsinogen and negative for type III mucin, were present gastrin receptor-null mouse. In contrast, in histamine H_2 receptor-null mice and double-null mice expression levels of pepsinogen per cell are very low and mature chief cells were very scarce. Considering the marked difference in pH values in histamine H_2 receptor-null mice and double-null mice (Fig. 6), the difference in chief cells in these mice is not attributable to low acidity but rather to disruption of the histamine H_2 receptor itself. Genetic ablation of parietal cells with $H^{(+)}/K^{(+)}$-ATPase promoter resulted in loss of mature chief cells, which can be taken as evidence that parietal cells are involved in chief cell maturation (Canfield et al., 1996; Li et al., 1996). However, it has been suggested

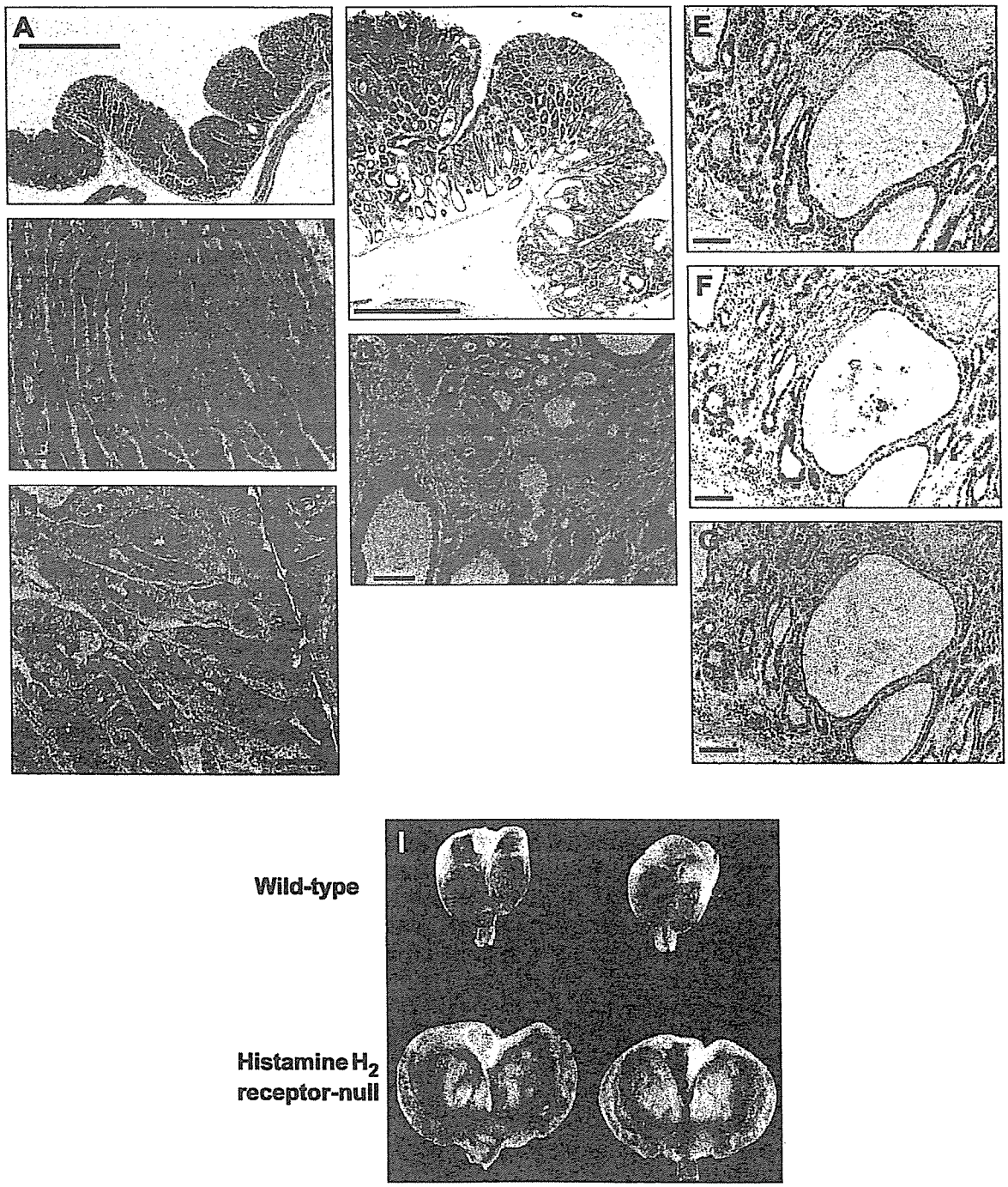

Fig. 7. Oxyntic mucosa from 12-month-old wild-type and histamine H_2 receptor-null mice. A, B, C, D, E, F, G and H Sections of oxyntic mucosa from histamine H_2 receptor-null mice (B, D, E, F, G, H) and wild-type (A, C) mice were stained with hematoxylin and eosin (A, B, C, D) and with anti-$H^{(+)}/K^{(+)}$-ATPase antibody (E), anti-pepsinogen antibody (F) or with anti-HDC antibody (G) and PAS (H). Arrows indicate interstitial cells. Scale bars, 1000 μm (A, B); 100 μm (E, F, G); 50 μm (C, D, H). (I) Macroscopic views of stomachs from wild-type and histamine H_2 receptor-null mice. The excised stomachs from 12-month-old mice were opened along the greater curvature.

that chief cell precursor cells express $H^{(+)}/K^{(+)}$-ATPase (Mutoh et al., 2002). Thus, it is likely that ablation of chief cell precursors rather than ablation of parietal cells resulted in the loss of chief cells observed in the study (Canfield et al., 1996; Li et al., 1996). In contrast, our pepsinogen and type III mucin findings show that the histamine H_2 receptor per se is involved in production and/or secretion of pepsinogen in chief cells. Thus, the histamine H_2 receptor is indispensable for chief cell maturation at least in terms of pepsinogen secretion.

Even in double-null mice, with severely impaired acid production, parietal cells and $H^{(+)}/K^{(+)}$-ATPase were present (Table 1). In addition, electron microscopic analysis of parietal cells from double-null, gastrin receptor-null and histamine H_2 receptor-null mice revealed no essential ultrastructural differences as compared to wild-type mice

(data not shown). Thus, there is no apparent structural alteration in gastric acid secretion mechanisms in double-null mice. However, gastric pH values were higher than in double-null mice than in the other three kinds of mice studied and were unresponsive even to carbachol. In histamine H_2 receptor-null mice, carbachol-induced acid production was mostly preserved (Fukushima et al., 2003; Kobayashi et al., 2000). Thus, considering the loss of the in vivo acid production response in gastrin receptor-null mice, acid production via cholinergic stimuli is largely dependent on the gastrin receptor. The finding that gastrin receptor disruption in histamine H_2 receptor-null mice, i.e. double-null mice, resulted in marked elevation of gastric pH (Fig. 6) reinforces the notion that gastrin receptors in parietal cells function in gastric acid secretion (Fukushima et al., 2003). In any case, it is noteworthy that disrupting histamine H_2 and gastrin receptors resulted in loss of response to secretagogues, even in terms of gastric pH, confirming the pivotal roles of these receptors in gastric acid production and secretion.

Recently, Ogawa et al. (2003) reported that findings in the stomachs of aged histamine H_2 receptor-null mice were compatible with Menetrier's disease. Menetrier's disease is characterized by hyperplasia of oxyntic mucosa which is attributable to hyperplasia of surface mucous cells and is often accompanied by hypoplasia of gland cells and low gastric acidity (Wolfsen et al., 1993; Yamada et al., 1995). As we previously reported, oxyntic mucosa from histamine H_2 receptor-null mice is characterized by marked hyperplasia of downward migrating cells, while hyperplasia of surface mucous cells is negligible (Fukushima et al., 2003). In 12-month-old mice, marked gastric mucosal hypertrophy was observed. However, as shown in Fig. 7, the extremely hypertrophic gastric mucosa consists of markedly elongated glands, cysts which originated from dilated gastric glands and increased interstitial tissues. The contribution of surface mucous cells is minimal. Thus, we consider it difficult to conclude that the gastric mucosal findings of aged histamine H_2 receptor-null mice are compatible with Menetrier's disease.

Rather, histological findings in aged histamine H_2 receptor-null mice can be fully explained by the findings in their 10-week-old counterparts. Oxyntic mucosal stem cells reside in the upper one-third of the mucosa away from the basal region and differentiate, growing upward or downward (Karam and Leblond, 1993a). In histamine H_2 receptor-null mice, marked hyperplasia of downward migrating cells results in unlimited movement of stem cells away from the basal region of the gastric mucosa (Fukushima et al., 2003). In addition, in the mid-portion of gastric glands both the number and mucous content of mucous neck cells are increased, which can lead to increased viscosity of the gastric juice retained in the mid-portions of gastric glands. Thus, due to this marked elongation of gastric glands together with the increased viscosity of gastric juice, gastric glands in histamine H_2 receptor-null mice would presumably be susceptible to occlusion. Once occlusion occurs, secretions from gland cells, even if impaired, promote the formation of cysts. Since gastric pH values per se are essentially preserved in histamine H_2 receptor-null mice (Fukushima et al., 2003; Kobayashi et al., 2000), leakage of contents and cystic rupture are expected to induce inflammation and an increase in interstitial tissues. Therefore, although the phenotype of stomachs from aged histamine H_2 receptor-null mice appears to be quite unusual, there is no essential difference between gastric mucosae from young and aged histamine H_2 receptor-null mice.

In conclusion, we have used double-null mice to show that (1) gastrin and histamine H_2 receptors are both essential in gastric acid production and secretion, (2) the histamine H_2 receptor plays a pivotal role in chief cell maturation, (3) gastrin gene products other than gastrin-17, such as glycine-extended gastrin, might be involved in surface mucous cell proliferation and (4) hypertrophy of gastric mucosa from histamine H_2 receptor-null mice is due to hyperstimulation of gastrin receptors via marked hypergastrinemia. Since gastric oxyntic mucosa is quite unique in that different cell types interact with each other both structurally and functionally, our murine models are potentially valuable for further analyzing differentiation of gastric mucosa and gastric acid secretion mechanisms.

Acknowledgments

This research was supported in part by a grant (to T. Saitoh and T. Ishikawa) from the Ministry of Education, Culture, Sports, Science and Technology, Japan. We are grateful to Ms. Masako Fujita, Ms. Kazuyo Shirai and Ms. Manami Ikematsu for helping with our experiments in this study.

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Seronegative Alpha-Fetoprotein-Producing Gastric Cancer: An Early Form of Aggressive Cancer

Key words: Alpha-fetoprotein, gastric cancer, metastasis, endoscopic mucosal resection

Alpha-fetoprotein (AFP)-producing gastric cancer (AFP-GC) represents an unusual form of aggressive adenocarcinoma, and comprises 2–9% of cancers derived from stomach epithelia. AFP-GC displays a complex histological phenotype, and hepatoid or enteroblastic differentiation may occur, although this is by no means obligatory (1, 2). Lectin column analyses of AFP synthesized by such tumors has revealed that AFP-GCs are not always derived from hepatoid differentiation of the foregut; rather, these gastric carcinomas might be categorized as medullary tumors producing gastrointestinal tract-specific AFP. The cadherin family is deeply involved in establishment of the histological structure of cells derived from epithelia (3). In gastric cancer cells, E-cadherin is predominantly expressed. However, in a certain population of AFP-GCs, expression of E-cadherin is absent, replaced by the expression of N-cadherin. This finding is in good accord with the phenotypic diversity of AFP-GC, which may reflect the origin of the cancer as an aggressive clone through genetic progression and/or divergence. Indeed, loss-of-heterozygosity (LOH) analysis using microdissected samples of AFP-GCs has revealed heterogeneous patterns of LOH (4).

AFP-GC is characterized by frequent serosal invasion, lymph node invasion and liver metastasis, and offers a very poor prognosis compared with more common gastric cancers (5). Differential diagnosis should seek to exclude metastasizing germ cell tumor. An immunohistochemical study has revealed characteristics comprising high proliferative activity, weak apoptosis, and rich neovascularization in this tumor (6). The causal relationship between AFP production and high malignant potential of AFP-GC remains unclear. A recent study clearly indicated that rather than methylation of the AFP promoter region, the absence of AT-motif binding factor 1 (ATBF1), a repressive transcription factor for the AFP gene, is responsible for the AFP-expressing phenotype, and absence of ATBF1 represents a distinct characteristic of AFP-GC (7). However, whether or not the absence of ATBF1 might also be important for the highly malignant nature of the tumor is still unclear. One explanation for the high malignant potential of AFP-GC may be aberrant expression of a growth factor and receptor system. Hepatocyte growth factor (HGF) and its receptor, c-Met, are known to mediate mitogenic and motogenic signals for epithelial cells in various organs, including the stomach (8). HGF is a potent growth factor on gastric epithelia, and may be involved in the promotion of tumor progression. A higher frequency of c-Met expression has been observed in AFP-GC than in other types of gastric cancer (9). In addition, a higher expression of an isoform of vascular endothelial growth factor, VEGF-C, and P-glycoprotein (P-gly) has also been reported in AFP-GC (10, 11). VEGF-C is considered to be associated with tumor progression through angiogenic or lymphangiogenic function, while P-gly is thought to be responsible for the phenotypic expression of multidrug resistance. These factors, together with other as-yet-unidentified factors, are probably involved in the high invasiveness and metastatic potential of AFP-GC.

As mentioned above, tumors are often advanced and complicated by liver metastases at the time of diagnosis. Surgical resection of AFP-GC is thus often unsatisfactory; 75% of cases are in stage III or IV disease when detected, and surgery is non-curative in 48% of operated patients (12). The 5-year survival rate and median survival period in all patients is 22% and 14 months, respectively, compared to 42% and 29 months, respectively, in patients with curative gastrectomy (6). However, several case reports have indicated that long-term survival can be achieved when patients with stage I or II tumor undergo curative gastrectomy. In the present issue of Internal Medicine, Hirasaki et al report a case of AFP-GC treated using endoscopic mucosal resection (EMR) and additional surgery (13).

See also p 926.

This case is quite important, in that no sign of lymph node involvement was noted preoperatively on diagnostic imaging including computed tomography, and treatment with EMR appeared successful. However, histological analysis of the resected specimen revealed submucosal and vascular invasion. Additional surgery was thus performed and micrometastases of the resected lymph nodes were detected. Based on histological findings in the resected region, namely the presence of cells with a clear cytoplasm and hyperchromic round nuclei, the authors considered the possibility of AFP-GC, although serum AFP levels were not elevated. Immunohistochemistry using anti-AFP antibody confirmed this prediction, and seronegative AFP-GC was diagnosed.

This case report underlines the importance of paying attention to this type of gastric cancer, which displays aggressive behavior and clinical and biological features quite different from typical gastric cancers. To control this highly invasive gastric cancer, tactics for early detection and novel multimodal therapies are badly needed.

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American Journal of Epidemiology Advance Access published July 7, 2006

American Journal of Epidemiology

DOI: 10.1093/aje/kwj232

Original Contribution

Prospective Cohort Study of the Risk of Prostate Cancer among Rotating-Shift Workers: Findings from the Japan Collaborative Cohort Study

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Received for publication September 2, 2005; accepted for publication March 7, 2006.

Shift workers have been reported to have an increased risk of some cancers. However, the risk of prostate cancer in shift workers is not known to have been examined previously. This study prospectively examined the association between shift work and risk of prostate cancer incidence among 14,052 working men in Japan enrolled in a large-scale prospective cohort. A baseline survey was conducted between 1988 and 1990. Subjects were asked to indicate the most regular work schedule they had undertaken previously: day work, rotating-shift work, or fixed-night work. During 111,974 person-years, 31 cases of prostate cancer were recorded. The Cox proportional hazards model was used to estimate the risk, with adjustments for age, family history of prostate cancer, study area surveyed, body mass index, smoking, alcohol drinking, job type, physical activity at work, workplace, perceived stress, educational level, and marriage status. Compared with day workers, rotating-shift workers were significantly at risk for prostate cancer (relative risk = 3.0, 95% confidence interval: 1.2, 7.7), whereas fixed-night work was associated with a small and nonsignificant increase in risk. This report is the first known to reveal a significant relation between rotating-shift work and prostate cancer.

circadian rhythm; cohort studies; Japan; occupational exposure; prostatic neoplasms; work schedule tolerance

Abbreviations: CI, confidence interval; JACC, Japan Collaborative Cohort; RR, relative risk.

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workers and fixed-night workers compared with day workers (21). In the analysis, relative risks were adjusted for age only and for age, study area, and family history of prostate cancer. Relative risks were further adjusted for the lifestyle-related factors and the occupational and psychosocial factors listed in table 1. Missing values for adjusting variables were treated as an additional category. The assumptions for the Cox proportional hazards model were checked by using the goodness-of-fit testing approach and were found to be valid. Calculations were performed with Stata version 8.0 (Stata Corporation, College Station, Texas) and SPSS version 12.0 (SPSS, Inc., Chicago, Illinois) statistical software.

Approval

This study was approved by the Ethics Committee for Medical Care and Research, University of Occupational and Environmental Health, Japan, and the Ethical Board of the Nagoya University School of Medicine, Japan.

RESULTS

Table 1 shows the baseline characteristics of the study subjects according to type of work schedule. Of the 14,052 men, 11,269 (80.2 percent) reported day work, 982 (7.0 percent) reported fixed-night work, and 1,801 (12.8 percent) reported rotating-shift work. Compared with those who had engaged in day work, men who had performed rotating-shift work reported a higher prevalence of high body mass index (>23.9 kg/m^2; 34.4 percent vs. 30.8 percent), sitting work (48.4 percent vs. 28.1 percent), and indoor work (44.5 percent vs. 38.6 percent) and a lower prevalence of office work (14.3 percent vs. 22.7 percent). The prevalences of family history of prostate cancer (0.8 percent vs. 0.3 percent), current smoking (55.4 percent vs. 53.8 percent), current alcohol drinking (75.6 percent vs. 78.0 percent), frequent stress (15.3 percent vs. 13.2 percent), higher educational level (higher than high school; 51.1 percent vs. 52.3 percent), and marriage (89.3 percent vs. 90.7 percent) revealed no differences in magnitude.

Table 2 shows the results of the Cox proportional hazards model analysis. Compared with that for the day workers, the age-adjusted relative risk for the rotating-shift workers revealed a significant increase (RR adjusted for age = 3.0, 95 percent CI: 1.2, 7.3). For the fixed-night workers, a slight increase was observed but was not significant (RR adjusted for age = 1.7, 95 percent CI: 0.5, 5.9). Adjustments for study area, family history of prostate cancer, body mass index, smoking, alcohol drinking, job type, physical activity at work, workplace, perceived stress, educational level, and marriage status did not significantly alter these results (rotating-shift work: RR adjusted for age, study area, and family history of prostate cancer = 2.5, 95 percent CI: 1.0, 6.2; RR adjusted for age, study area, family history of prostate cancer, body mass index, smoking, alcohol drinking, job type, physical activity at work, workplace, perceived stress, educational level, and marriage status = 3.0, 95 percent CI: 1.2, 7.7 and fixed-night work: RR adjusted for age, study area, and family history of prostate cancer = 1.5, 95 percent CI: 0.4, 5.3; RR adjusted for age, study area, family history of prostate cancer, body mass index, smoking, alcohol drinking, job type, physical activity at work, workplace, perceived stress, educational level, and marriage status = 2.3, 95 percent CI: 0.6, 9.2).

Table 3 shows the characteristics of the prostate cancer cases according to type of work schedule. Between day workers and rotating-shift workers, there were no differences in mean age at baseline (58.5 years vs. 59.3 years), mean number of years of follow-up (6.7 vs. 6.3), mean age at endpoint (65.2 years vs. 65.6 years), family history of prostate cancer (one case for each work schedule), and mean body mass index at baseline (22.8 kg/m^2 vs. 21.6 kg/m^2).

DISCUSSION

We found a significant increase in prostate cancer risk among rotating-shift workers. This result supports the hypothesis that shift work is a risk factor for prostate cancer. To our knowledge, this is the first report to reveal the association of rotating-shift work with prostate cancer.

The melatonin pathway, which is closely linked to circadian rhythms, is most frequently implicated in the observed increase in tumor incidence among shift workers. Secretion of the hormone is low during daytime, increases soon after the onset of darkness, peaks in the middle of the night, and gradually falls until morning. The hormone has been reported to affect circadian rhythms and exhibit both hypnotic and antineoplastic effects (9). Several possible mechanisms have been proposed regarding tumor growth inhibition by melatonin. Melatonin may suppress tumor growth by down-regulating transcription, secretion, or activity of growth factors; it may stimulate the immune system through increased production of interleukin-2 and interleukin-4 by T-helper cells; lastly, it may protect DNA against oxidative damage by scavenging free radicals (9). Among shift workers, it has been proposed that an elevated risk of cancer may be due to a phase shift and reduced secretion of melatonin, resulting from a disruption in circadian rhythms (22–24). In terms of prostate cancer, previous studies showed that melatonin could directly inhibit proliferation of cultured prostate cancer cells (25). The melatonin pathway may therefore be relevant to prostate cancer incidence among shift workers.

The effect of sex hormones may be secondary to melatonin. Melatonin suppression is believed to increase the level of sex hormones (9, 24). Among female shift workers, increased levels of estradiol and low levels of melatonin have been reported (22). An interrelation between melatonin and testosterone has also been suggested for males (26), so high levels of testosterone due to low levels of melatonin could also be hypothesized in male shift workers. The growth and differentiation of the prostate is under androgen control (12); therefore, this pathway should attract attention as a relevant mechanism.

Decreased exposure to daylight is known to be a risk factor for prostate cancer (27, 28). This link was explained by decreased production of vitamin D due to reduced exposure to ultraviolet rays. The biologically active form of vitamin D, 1α,25-dihydroxyvitamin D_3, has been reported

TABLE 2. Relative risk of prostate cancer associated with work schedule, Japan Collaborative Cohort Study for Evaluation of Cancer Risk, 1988–1997

Work schedule	No. of person-years	No. of cases	Age adjusted			Multivariate adjusted					
			RR*	95% CI*	*p* value	RR†	95% CI	*p* value	RR‡	95% CI	*p* value
Daytime	89,179	21	1.0			1.0			1.0		
Fixed night	8,272	3	1.7	0.5, 5.9	0.387	1.5	0.4, 5.3	0.534	2.3	0.6, 9.2	0.231
Rotating shift	14,523	7	3.0	1.2, 7.3	0.016	2.5	1.0, 6.2	0.043	3.0	1.2, 7.7	0.020

* RR, relative risk; CI, confidence interval.
† Adjusted for age, study area, and family history of prostate cancer.
‡ Adjusted for age, study area, family history of prostate cancer, body mass index, smoking, alcohol drinking, job type, physical activity at work, workplace, perceived stress, educational level, and marriage status.

to inhibit proliferation of prostate cancer cells (29, 30). In shift work, daylight exposure is shortened, so elevated risk could be explained through this pathway. In our studies, however, rotating-shift workers, whose exposure to daylight is longer, had a higher risk compared with fixed-night workers, whose exposure is relatively shorter. Effects on circadian rhythms were suggested to be more serious for rotating-shift workers compared with fixed-night workers (31). Disrupting the circadian rhythm in rotating-shift workers may therefore have a greater effect on tumorigenesis than shortening exposure to daylight.

The scientific field of chronotoxicology examines the biologic cycle of susceptibility to chemical toxicity (32). The toxicity of many chemical agents was reported to vary with circadian change, and the possibility was suggested that susceptibility to chemical agents may increase at night in humans (4, 32). Occupational exposure to chemical agents such as cadmium and manganese is suspected to increase the risk of prostate cancer (33, 34), so these agents may have a stronger influence among shift workers. Exposure to these chemicals is considered rare, however. Thus, the relevance of chemical toxicity to this study is questionable.

Diet is also known to play a role in prostate cancer. A Western diet, which is relatively high in fat and meat, may contribute to higher prostate cancer risk (12). We examined confounding dietary factors, including meat, vegetables, fried foods, milk, and butter, but adjustment for these covariates did not alter the results (data not shown).

An association between prostate cancer and obesity has not been established in spite of repeated studies. However, obesity has been hypothesized to be a risk factor for prostate cancer because of the connections between body size and testosterone (35). Shift workers are known to be a high-risk group for obesity (4). In the current study population, rotating-shift workers showed a higher distribution of the highest body mass index compared with daytime workers (table 1). High body mass index among shift workers might confound the result. However, there was no significant difference in baseline body mass index between the prostate cancer cases in day work and in rotating-shift work (table 3). In addition, the significant association between rotating-shift work and prostate cancer was still observed after we adjusted for body mass index. Therefore, the effect of obesity, or at least body mass index at the time of the baseline survey, on the current study appears limited.

Limitations of our study

Important limitations of our study should be discussed. First, in terms of ascertaining the risk of prostate cancer, the follow-up time was short and the cohort was relatively small. Although this study had enough power to show the

TABLE 3. Characteristics of prostate cancer cases according to type of work schedule, Japan Collaborative Cohort Study for Evaluation of Cancer Risk, 1988–1997

Characteristic	Work schedule			*p* value
	Daytime	Fixed night	Rotating shift	
No. of cases	21	3	7	
Death certificate only	1	0	0	
Mean age in years at baseline (standard deviation)	58.5 (6.4)	53.7 (4.0)	59.3 (5.4)	0.39*
Mean no. of years of follow-up (standard deviation)	6.7 (3.0)	5.6 (2.6)	6.3 (2.5)	0.82*
Mean age in years at endpoint (standard deviation)	65.2 (6.6)	59.3 (1.8)	65.6 (4.8)	0.27*
No. of cases with a family history of prostate cancer	1	0	1	0.60†
Mean body mass index‡ at baseline (standard deviation)	22.8 (2.0)	21.9 (0.9)	21.6 (2.0)	0.33*

* Derived from analysis of variance.
† Derived from the chi-square test.
‡ Weight (kg)/height $(m)^2$.

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文献レビューによる胃がん検診の受診率向上対策

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はじめに

胃がん検診の受診率向上対策を明らかにすることを目的として、胃がん検診の受診率について検討した内外の論文をレビューしたので、その結果を以下に記述する。

なお、大腸がん検診についても同時に検討したが、これについては他誌において発表することとなっているのでここには記載しない。

対象と方法

MEDLINE、CINHAL、医学中央雑誌、日本消化器集団検診学会雑誌及び日本消化器内視鏡学会雑誌の1985年1月～2005年2月の期間の胃がん検診関係論文を検索し、胃がん検診の受診率について報告された論文を選び出した。これらの論文の中で受診率向上対策が検討されているものと 受診者の要因調査についての論文を最終的な検討対象とした。

Key Question として、「日本における胃がん検診受診率を向上させるにはどうすれば良いか？」を設定した上で、Check List として1）目的は明確か（受診率の向上が目的とされているか)、2）解析方法は無作為化比較対照試験か観察的手法か、3）対象集団の定義と人数、4）介入群と対照群は明確に定義されているか、5）評価指標は明確に定義されているか、6）結果、7）研究の限界や偏り（Bias）が記載されているか、8）結論、9）結論は適切に評価されているか、10）日本において適用可能かの10項目について整理した。

結　果

胃がん検診関係論文の検索の結果、胃がん検診の受診率について報告された論文は、27論文（英語論文1編、日本語論文26編）あった。これらの論文のうち、受診率向上対策についての3論文[1)-3)]と受診者の要因調査についての5論文[4)-8)]の合計8論文を検討対象とした。これら以外の19論文は、単なる実施報告のものが5論文、受診状況に関する調査についてのものが6論文、受診率の測定法についてのものが2論文、経済評価についてのものが3論文及びその他が3論文となっていた。

これらの8論文のそれぞれについて Check List の10項目を整理して表1～表8に示す。

表1

著者	今井貴子、他
論文名	群馬県都市部における胃集検推進方式について ── 前橋市医師会方式：胃癌個別検診について ──
発表雑誌	消化器集団検診、88号、56-62、1990
目的（受診率向上）は明確か？	前橋市で実施されていた胃癌個別検診とチケット制の組合せ法を他都市部の車検診法と比較して、その長短所を明らかにすること。
解析方法（RCT／観察的）は？	観察的方法（無作為化されていない集団と集団の比較）
対象集団の定義？　人数？	対照群（高崎市）と介入群（前橋市）、ともに人口約30万人
介入群と対照群は明確に定義されているか？	対照群：群馬県高崎市；S61年に精密検査が医師会委託となったが一次検診は車検診のままであった、介入群：群馬県前橋市；S59年より個別検診導入、S62年チケット制導入
評価指標は明確に定義されているか？	対照群（高崎市）と介入群（前橋市）と間の受診率の比較、介入群（前橋市）におけるチケット制導入前後での受診率の比較。なお、受診率の分母の定義は示されていない。
結果	受診率は、対照群（高崎市）：S58年3.1%、S59年4.9%、S60年6.0%、S61年4.0%、S62年5.5%、S63年6.7%、介入群（前橋市）S58年1.7%、S59年7.8%、S60年8.9%、S61年10.5%、S62年15.7%、S63年13.6%
研究の限界が記載されているか？ Biasは？	初回受診者の割合が不明であり、統計学的処理もされていないので偶然の結果であったかどうかも明確でない点で一般化することができないと考えられるが、そのような記載はなかった。
結論	個別検診とチケット制が導入された前橋市と車検診のままであった高崎市の受診率（検診率）の間で2倍の差が生じた。
結論は適切に評価されているか？	統計学的処理がなされていないので不適切と考えられる。
日本において適用可能か？	住民の多様な要望に対応できるという点については、日本での研究であり他の日本の地域においても適応可能と考えられる。

RCT：無作為化比較対照試験

表2

著者	河村奨、他
論文名	胃集検受診率向上へ寄与するもの ── 複合検診と直接撮影方式 ──
発表雑誌	消化器集団検診、87号、11-19、1990
目的（受診率向上）は明確か？	検診の技術面としての検診システム、検査方法が胃集検受診率にどう影響しているかを検討した。
解析方法（RCT／観察的）は？	観察的方法（市町村・保健所別の受診率の年次推移の観察）
対象集団の定義？　人数？	山口県内の市町村・保健所別受診率の推移の観察と従業員約900人の化学工業系の職域
介入群と対照群は明確に定義されているか？	胃がん検診と腹部超音波検査や基本健康診査等の併用の導入をを介入として、導入前後での比較を行っており、導入前が対照となっている。併用方法には様々な類型があり介入の定義は不明確である。
評価指標は明確に定義されているか？	受診者数、受診率が中心であるが、胃がん発見率も示されている。なお、受診率の分母の定義が明確でない。
結果	胃集検単独よりも複合検診の方が5－10%程度受者の増加が見られた。
研究の限界が記載されているか？ Biasは？	記載されていない。
結論	他のがん検診や基本検診と併せて胃がん検診を実施する複合検診は、胃集検受診者増に寄与している。
結論は適切に評価されているか？	統計学的検討がなされていないので偶然の結果であることを否定し得ない。この点で適切とは言えない。直接撮影法による胃がん検診にもふれられているが、きちんとした評価がされていない。（導入前の受診者数が示されていない。）
日本において適用可能か？	他のがん検診や基本検診と併せて胃がん検診を実施することは、論文の結果のいかんに関わらずより望ましいと考えられ、他の地域でも適応されている。

表3

著者	大滝隆子、他
論文名	川崎市民の胃がん検診の受診行動に関する調査
発表雑誌	消化器集団検診、35巻1号、89-93、1997
目的（受診率向上）は明確か？	市民が胃がん検診に関してどう行動しているかを把握する。
解析方法（RCT／観察的）は？	自記式調査の解析（過去3年間の胃の検査の受診についての横断調査）
対象集団の定義？　人数？	性・年齢・行政区で層化無作為抽出された40歳以上の川崎市民3,000人
介入群と対照群は明確に定義されているか？	介入についての研究ではないので設定されていない。
評価指標は明確に定義されているか？	過去3年間のそれぞれの年の胃X線検査と上部内視鏡検査のそれぞれの受診率（受診者数／回答者数）
結果	回収は1,257人（回収率41.9%）。X線検査受診歴ありは85.6%であり年齢に関係なく80%を越えていた。X線検査の28.8%は医療として受診、残りの71.2%は検診として受診、うち市の検診は全体の16.8%。過去3年のいずれでもX線検査のみ受診約32%、内視鏡検査のみ受診約2%、両方受診約8%、合計約42%。過去3年間毎年受診28%、過去3年間1回以上受診61.2%。未受診の主たる理由は必要性を感じなかったため（60.5%）。
研究の限界が記載されているか？Biasは？	41.9%の回答者における受診率であり、バイアスの影響を受け高く出ている点にふれている。
結論	地域胃集検受診者数の減少は、職域検診の普及と集検以外の個別検診の充実による。
結論は適切に評価されているか？	状況証拠としては適切な推論と考えられるが、直接的根拠としては弱いと思われる。
日本において適用可能か？	結果から、どうすれば受診率を向上させることができるかということは導き出せない。

表4

著者	赤松直樹、他
論文名	住民調査からみた胃集検受診の実態第一報　胃集検受診に関連する要因
発表雑誌	産業医大誌　8(2)、177-183、1986
目的（受診率向上）は明確か？	胃集検受診の関連要因のみで、受診率向上に言及していない
解析方法（RCT／観察的）は？	縦断的研究
対象集団の定義？　人数？	40歳以上の全住民3,660人（回答者2,404、回収率65.7%）
介入群と対照群は明確に定義されているか？	介入についての研究ではないので設定されていない。
評価指標は明確に定義されているか？	過去3年間の胃集検受診率（3年間に1度）
結果	検診受診率は男性24.1%、女性14.1%で男が有意に高かった。これは職域検診の影響によるもので、公務員の受診率は78.7%、無職の受診率は12.5%であった。受診に強く関連する因子として、家族のがん既往歴がもっとも大きかった。
研究の限界が記載されているか？Biasは？	回答率が低い。論文の中ではあまりふれられていない。
結論	受診率向上のために「家族のがん既往歴よりも強い動機付けが必要」という結論であった。
結論は適切に評価されているか？	受診率向上対策に直接結びつけられるものではない。
日本において適用可能か？	日本における調査であり、応用は可能。

表5

著者	坪野吉孝、他
論文名	地域胃がん検診の受診行動の心理的規定要因 ── Health Belief Model による検討
発表雑誌	日本公衆衛生学会誌、40(4)、255-264、1993
目的（受診率向上）は明確か？	胃集検受診の心理学的分析である。受診率向上対策は考察の中で例示している。
解析方法（RCT／観察的）は？	アンケートを用いた後ろ向き観察研究
対象集団の定義？　人数？	45－64歳の男女で老人保健法による胃がん検診対象者627人中、基本健康診査受診者400人、ここから無効回答を除外した337人。さらに1年前に行った EPQ-R による性格調査の回答者278人を対象とした。
介入群と対照群は明確に定義されているか？	介入についての研究ではないので設定されていない。
評価指標は明確に定義されているか？	過去5年の胃がん検診受診回数
結果	健康への関心、胃がんに罹患する危険性の意識、胃がん検診の効果の認識が、胃がん検診の受診回数と正の相関。胃がんの深刻さの認識、胃がん検診受診上の障害の意識が負の相関
研究の限界が記載されているか？ Bias は？	Health Belief Model（HBM）の尺度の信頼性、後ろ向き研究であるデザイン上の問題。HBM の理論的妥当性
結論	HBM が、胃がん検診の受診行動を理解する理論的支柱となる可能性を示唆する。
結論は適切に評価されているか？	受診率向上対策に直接結びつけられるものではないが、胃がんの深刻さの認識を増長することはかえって受診率低下を来す可能性を示唆するなど、参考になる部分はある。
日本において適用可能か？	日本における調査であり、応用は可能。

表6

著者	北　昭一、他
論文名	胃集検の精検受診率向上に関わる要因についての検討
発表雑誌	日本消化器集検学会誌、36(4)、461-467、1998
目的（受診率向上）は明確か？	精検受診率向上のための経験談
解析方法（RCT／観察的）は？	解析ではなく、実体験のレポート
対象集団の定義？　人数？	定義されていない
介入群と対照群は明確に定義されているか？	定義されていない
評価指標は明確に定義されているか？	定義されていない
結果	要精検者を減少させることで、精検受診率は100%近くなった。受診勧奨は、検診の現場で医師が直接行っている。
研究の限界が記載されているか？ Bias は？	記載されていない
結論	日程計画、技師の撮影能力、医師の読影能力などが有機的に機能しないと、精検受診率は向上しない。
結論は適切に評価されているか？	精検受診率向上対策であり、検診受診率ではない。
日本において適用可能か？	日本の研究である。