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Sequential alterations in gastric biopsies and tumor tissues support the multistep process of carcinogenesis

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Summary. Gastric cancer is the second leading cause of cancer related death worldwide. In the UAE, recent data show an increase in the number of patients with gastric cancer highlighting the need for greater understanding of its pathogenesis. Gastric cancer is generally believed to develop on a background of chronic atrophic gastritis which eventually leads to intestinal metaplasia, dysplasia and finally invasive carcinoma. Recently this multistep process of carcinogenesis has been challenged. Therefore, the aim of this study is to define alterations in antral mucosal biopsies and cancer tissues to investigate whether they could be used to assemble a tissue array supporting the multistep model of carcinogenesis. Gastric mucosal tissues were obtained from informed individuals undergoing endoscopy (for upper gastrointestinal symptoms) and gastrectomy (for adenocarcinoma) in Tawam Hospital. All tissues were processed for microscopic examination. Eighty nine antral biopsies were categorized as: normal (33%), mild superficial gastritis (34%) and severe atrophic gastritis (33%). About 5% of the latter exhibited evidence of intestinal metaplasia. Cancer tissues obtained from three patients were microscopically examined in three regions: safe resected margin, tumor edge and tumor center. Progressive changes in mucosal thickness, dysplasia and cellular transformation were observed, and when compared with alterations in biopsies, all appeared to represent a continuum of progression toward invasive adenocarcinoma. In conclusion, the tissue array presented in this study supports the multistep process of gastric carcinogenesis and will be helpful in examining the expression pattern of tumor markers or molecules that could help in the early detection of gastric cancer.

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Introduction

Gastric cancer is considered to be one of the leading global causes of death from malignant disease. It accounts for approximately 10% of all newly diagnosed cancer cases in some areas of the world (Roder, 2002). Despite a recent gradual decline in the incidence and mortality rate of gastric cancer in some countries, it remains the fourth most common cancer and the second leading cause of cancer related death worldwide (Brenner et al., 2009; Ferlay et al., 2010). In the United Arab Emirates, data kindly provided by National Cancer Registry at Tawam Hospital show a decreasing trend in the number of reported cases from 2002 till 2005. Then, in 2006 and 2007, there was an increase in the number of cases, an alarm call to increase efforts for basic research and to improve early diagnostic and therapeutic modalities.

The high frequency and mortality of gastric cancer are linked to lack of screening and early detection programs, leading to late diagnosis (Hohenberger and Gretschel, 2003). If gastric cancer is diagnosed at an early stage, patients can be treated endoscopically with submucosal resection/dissection, laparoscopically, or subjected to an open procedure (Nomura and Kaminishi, 2007). Unfortunately, there is no effective life saving treatment for most patients with advanced late-diagnosed gastric cancer. Preoperative (neoadjuvant) chemotherapy is in widespread use to downstage or reduce the burden of disease. Thus, there is a need for basic research to better understand the pathogenesis of gastric cancer at its early stages.

Over 95% of gastric malignancies are

adenocarcinomas. The remaining 5% are lymphomas, carcinoids and stromal tumors (Miller et al., 2005; Smith et al., 2006). According to Lauren's classification, gastric adenocarcinomas include: 1) undifferentiated or diffuse type (30-35%) which is common in low-risk young patients and is primarily located in the corpus region, and 2) differentiated or intestinal type (50-65%) which predominates in high-risk older individuals and is commonly located in the pyloric antrum (Lauren, 1965; Senapati et al., 2008).

The pathogenesis of intestinal adenocarcinoma is not well understood. However, as the name indicates, it has some features relating to intestinal mucosa. Therefore, during the process of cancer development, some areas of the normal gastric epithelium are replaced by well defined intestinal epithelium with scattered goblet cells. The underlying and adjacent stroma often harbors cellular elements of chronic gastritis: lymphocytes and plasma cells. Hence, it is believed that the progression of intestinal adenocarcinoma occurs in a multistep process, which can be described as a series of sequential phases. It develops with superficial gastritis, atrophic gastritis, intestinal metaplasia, dysplasia, and finally invasive carcinoma (Correa, 1991, 1992; Correa et al., 2006). A more or less similar multistep model of gastric carcinogenesis was also observed in various mouse models of gastric carcinogenesis (Fox and Wang, 2007; Karam, 2010).

The human stomach includes two main glandular regions. The oxyntic region (fundus plus corpus) is lined by a single layer of epithelial cells which form numerous pit-gland units. Each unit is populated by five main cell types: surface mucous or pit cells, mucous neck cells, pepsinogen-secreting zymogenic cells, acid-producing parietal cells, and enteroendocrine cells. In addition, a few small progenitor cells are found at the pit-gland junction (isthmus) which are responsible for the continuous renewal of the whole epithelium (Karam et al., 2003). No undifferentiated granule-free (stem) cells similar to those previously described in the mouse stomach (Karam and Leblond, 1993) were identified in the human oxyntic pit-gland units.

In the human pyloric antrum, the pit-gland units are shorter than those of the oxyntic region and are lined by two main mucus-secreting cell types: pit and gland cells. Even though the gastric antrum is the site for gastritis, which commonly leads to intestinal metaplasia and adenocarcinoma, there are very limited data concerning the epithelial features in this region of the human stomach, particularly during development of carcinoma. It is also not known whether these changes support the multistep process of carcinogenesis which has even been challenged in some studies (Testino et al., 2002; Kaminishi, 2005; Testino, 2006). The aim of this study is to examine mucosal biopsies and cancer tissues obtained from the pyloric antrum of informed patients to define the alterations that occur in these tissues and investigate whether these samples could be used to assemble a tissue array representative of the multistep process of gastric carcinogenesis.

Materials and methods

The design and protocol of this study were approved by the Ethics Committees for Research on Human Subjects in the Faculty of Medicine, UAE University and Tawam Hospital. Two groups of human tissues were examined in this study: gastric mucosal biopsies and gastric cancer tissues. Biopsies were taken during a period of two years from the pyloric antrum of fully informed patients (n=102) of both sexes ranging from 20-90 years of age. These patients were undergoing endoscopic examination in Tawam Hospital for investigation of upper gastrointestinal symptoms.

Cancer tissues were obtained from patients (n=3) following gastrectomy due to adenocarcinoma. Samples of cancer tissues were taken from three regions: tumor center, tumor edge and the safe (resection) margin i.e. the area at least 10 cm away from the tumor. Pyloric antral biopsies and cancer tissues from each region were processed for light microscopic examination.

From each patient undergoing endoscopy, at least one piece of tissue was processed for paraffin embedding. For cancerous lesions, at least five tissue pieces were processed from each of the three regions. Tissues with dimensions of at least 3x3 mm (biopsy) or about 10x5 mm (cancer) obtained from the patients were immediately immersed overnight (~24 hrs) in Bouin's solution. Fixed tissues were then dehydrated in ascending grades of alcohol, cleared in xylene and finally impregnated and embedded in paraffin. Tissue blocks were cut at 5 microns thickness and mounted on gelatin-coated slides.

For general histological analysis, some tissue sections from all samples were processed for routine staining with hematoxylin and periodic acid Schiff (PAS). Tissue sections were de-waxed in xylene and rehydrated with descending grades of alcohol. Sections were then incubated with periodic acid for 5 minutes and with Schiff's reagent for 15 minutes. Following a 10-minute wash in water tissue sections were dipped in hematoxylin twice and washed for 15 minutes. Finally, sections were dehydrated in alcohol, cleared in xylene and mounted with DPX medium. Stained tissue sections were examined with an Olympus BX41 light microscope and DP70 digital camera to define the histological features of the gastric mucosa in all samples.

Results

The biopsies obtained for the present study were all from individuals who were scheduled for endoscopic examination and shared only one feature, recurrent upper gastrointestinal symptoms. However, these individuals varied in their age, gender and *H. pylori* status (Table 1).

Microscopic examination of the gastric tissues obtained from mucosal biopsies and from the three regions of the resected cancerous stomachs revealed that

their structural features were highly variable.

Morphological features of pyloric antral biopsies of patients with upper GI symptoms

Analysis of the gastric mucosal tissue sections obtained from various biopsies revealed that some samples (n=13) were inadequate due to small size representing only the superficial part of the mucosa. All the remaining biopsies (n=89) showed the whole thickness of the mucosa and detailed microscopic examination revealed features representative of four different conditions.

a) In many of these adequate biopsies (n=25), the mucosa was characterized by a continuous sheet of luminal PAS-positive surface mucous cells with numerous openings of pits lined by similar mucus-secreting cells (Fig. 1A). The pits tend to have a relatively wide lumen continuous, proximally, with the gastric lumen. Distally, each pit leads to a narrow opening of a simple tubular gland. More than one gland may open into a single pit. Counts in tissue sections revealed that about 30 mucous cells line each side of the pit. The pits were continuous with glands which were lined mostly by mucous cells and only a few enteroendocrine cells. The lower portion of the pits was characterized by the presence of a few mitotic cells (Fig. 1A) consistent with their potential of self renewal. The

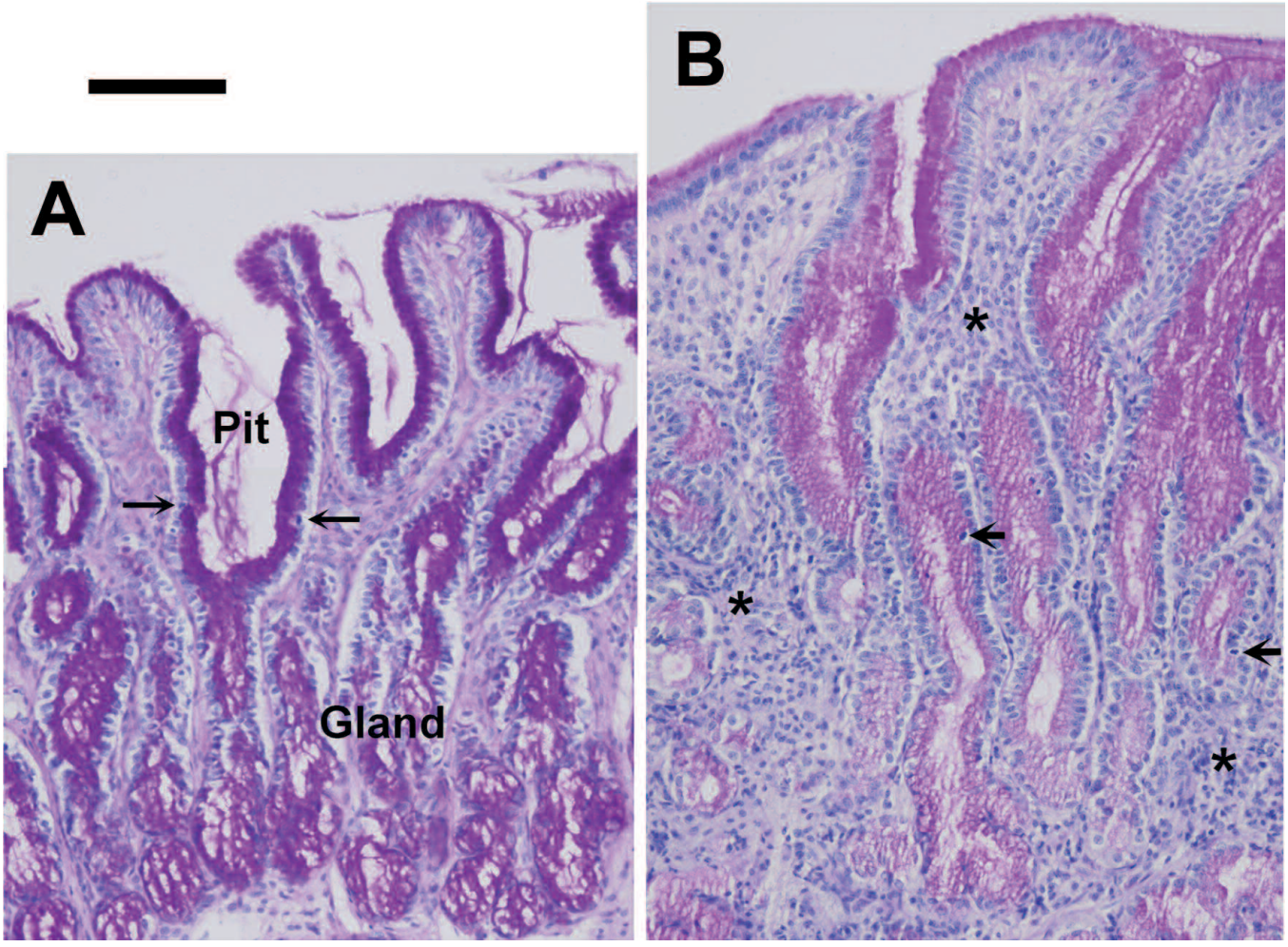

Fig. 1. Montages showing PAS- and hematoxylin-stained sections of the gastric mucosae as seen in biopsies of normal mucosa **(A)** and mild gastritis **(B)** obtained from the antrum of human stomachs. **A.** The mucosa is lined by a single sheet of epithelial cells which form numerous pits continuous with glands of similar length. The pit appears to have a lumen wider than that of the gland. The apical portion of the lining cells in the pits are stained darker with PAS than those of the gland cells. The connective tissue around pits and glands includes moderate amounts of cellular elements. The arrows point to mitotic figures which are commonly seen in the lower portion of the pits. **B.** The gastric mucosa as seen in an antral biopsy with mild gastritis. The mucous cells of pits and glands are stained purple with PAS. Note that the connective tissue between the pits and glands is infiltrated with lymphoid cells which become numerous and condense in multiple areas (asterisks). Mitotic cells (arrows) are seen in the lower portion of the pit and the upper portion of the gland. Bar: 40 μm.

glands were almost equal to the length of the pits and tended to have a narrow lumen. There were about 25 cells per side of each longitudinally cut gland; slightly less than the number of pit cells, perhaps due to the enlarged size of gland cells as they reach the bottom of pit-gland units. The lamina propria underneath the

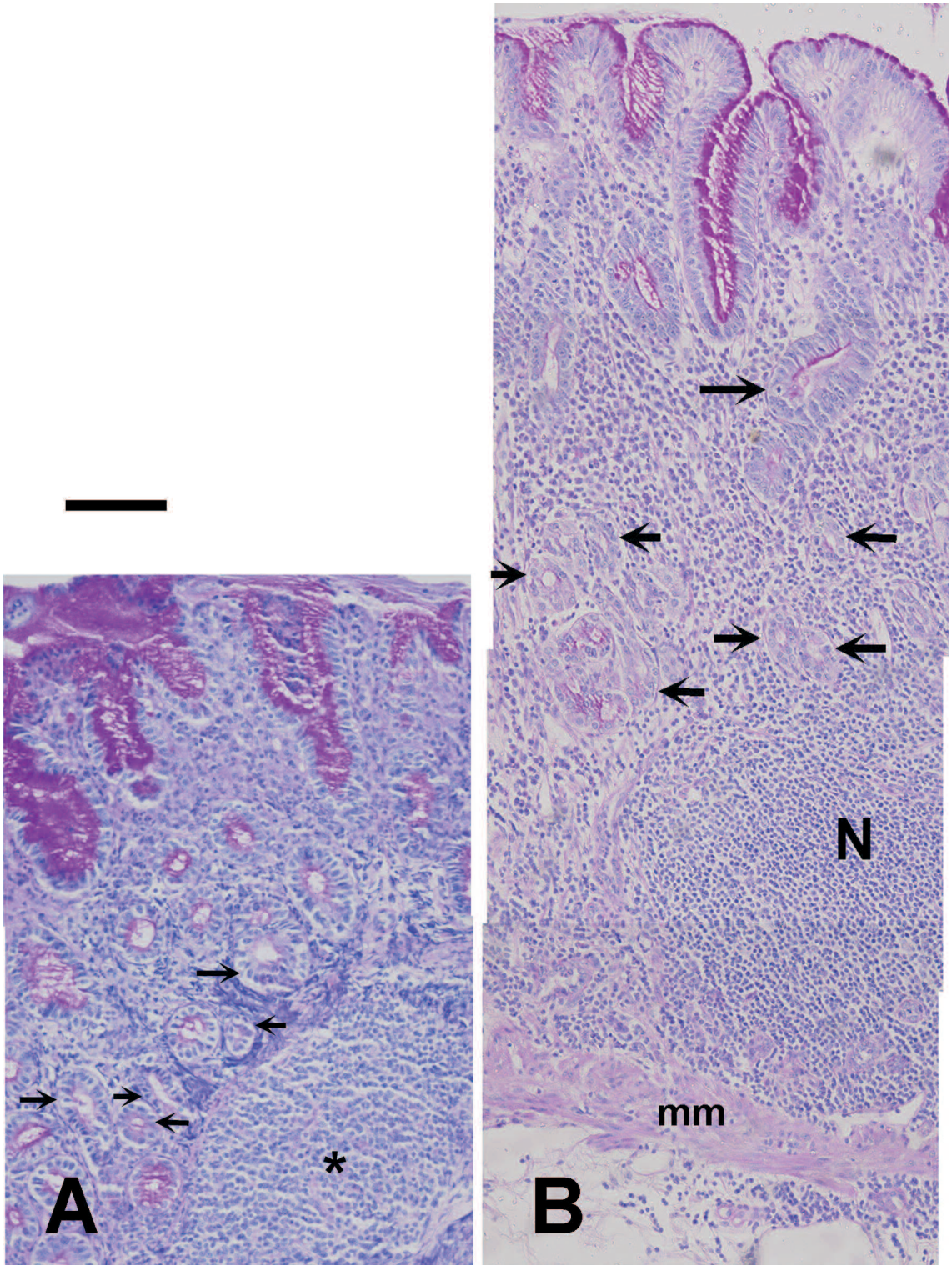

Fig. 2. The gastric mucosae of a biopsy showing severe chronic atrophic gastritis **(A)** and safe margin of resected cancer tissue **(B)**. **A.** Note the presence of a large aggregate of lymphoid cells forming a small nodule (asterisk) at the bottom. The remaining adjacent glandular profiles are lined with cells producing little PAS-labeled mucus (arrows) indicating their poorly differentiated state. **B.** In the "safe margin" of resected gastric cancer tissue, the upper half of the mucosa shows the gastric pits continuous with glandular profiles lined by poorly differentiated cells (arrows); some are seen in mitosis (upper arrow). In the basal half of the mucosa, there is a conglomeration of lymphoid cells which form a large lymphoid nodule (N) and also infiltrate through the muscularis mucosa (mm). Bar: 40 μm.

surface epithelium and between the pit-gland units was characterized by a moderate amount of connective tissue cells. All these features were considered to represent the gastric mucosa of control or healthy pyloric antrum. This group of individuals with healthy antral mucosae included more females than males, average age 35 years, and most of them tested negative for *H. pylori* (Table 1).

b) About one third of the biopsies (n=31) were characterized by increased thickness of the mucosa and diffuse infiltration of the connective tissue of the lamina propria with lymphoid cells (Fig. 1B). The gastric pits appeared slightly longer or hyperplastic, and mitotic cells containing little PAS-stained mucus were prominent in the middle of the pit-gland units (Fig. 1B). The lymphoid cells tended to aggregate in small multiple areas where interruptions of the glandular organization were observed. These features were suggestive of inflammatory reactions and these biopsies were considered to be representative of mild chronic gastritis. About two-thirds of this group of patients were females, average age 42 years, and most of them tested negative for *H. pylori* (Table 1).

c) In 30 biopsies there was a massive infiltration of the lamina propria with lymphoid cells (Fig. 2A). The integrity of the surface epithelium was disrupted. Thus, in multiple areas, the surface epithelium was absent and some glands were even atrophied. The remaining glandular profiles adjacent to the lymphoid cell aggregates were made of cells producing few mucous granules in the apical cytoplasm (like epithelial progenitors) as indicated by the linear PAS labeling at the luminal surface (Fig. 2A). These biopsies were taken to represent severe atrophic gastritis. Two-thirds of this group of severe gastritis patients were females, average age 45 years, and many of them tested positive for *H. pylori* (Table 1).

d) Only a small proportion of the biopsies (n=3) revealed evidence of intestinal metaplasia on a background of severe gastritis. In these tissues, multiple areas were observed in which the epithelial lining of the pits and the luminal surface included some scattered PAS-positive goblet cells surrounded by intestinal

Table 1. Diagnoses, numbers, demographic features, and H. pylori status of all subjects included in this study.

Diagnosis	Total	Age (years)			Gender		H. pylori			
		Mean	Min	Max	M	F	Positive	Negative	Eradicated	Not tested
Healthy	25	35	20	66	8	17	2	15	0	8
Mild gastritis	31	42	21	81	12	19	2	17	1	11
Severe gastritis	30	45	20	90	10	20	15	10	0	5
Metaplasia	3	48	44	52	1	2	0	3	0	0
Cancer	3	55	44	65	2	1	0	1	0	2

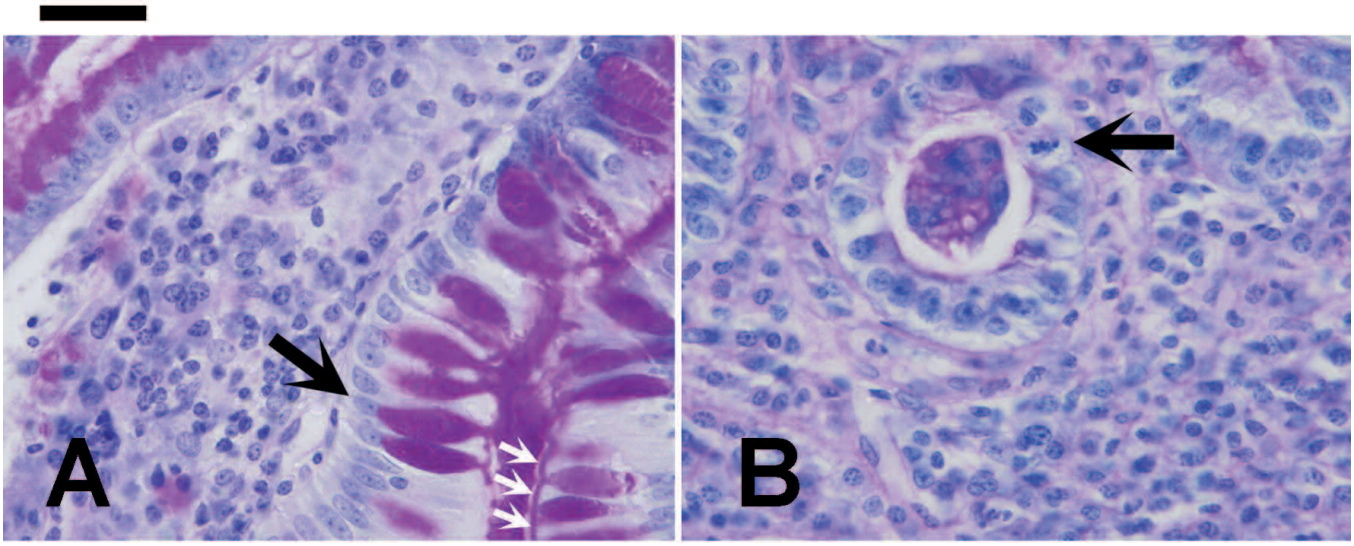

Fig. 3. Precancerous lesions as seen in some gastric mucosal biopsies and the safe resected margin of gastric cancer tissue. **A.** Intestinal metaplasia. Note the difference between the gastric epithelium, upper left, and the metaplastic epithelium with goblet (black arrow) and absorptive brush border cells (white arrows). **B.** Dysplasia. Note the dilated profile of gastric gland with a lumen filled with dead cells and epithelial lining consisting of small poorly differentiated cells showing little apical PAS labeling and evidence of mitosis (arrow). Bar: 40 μm.

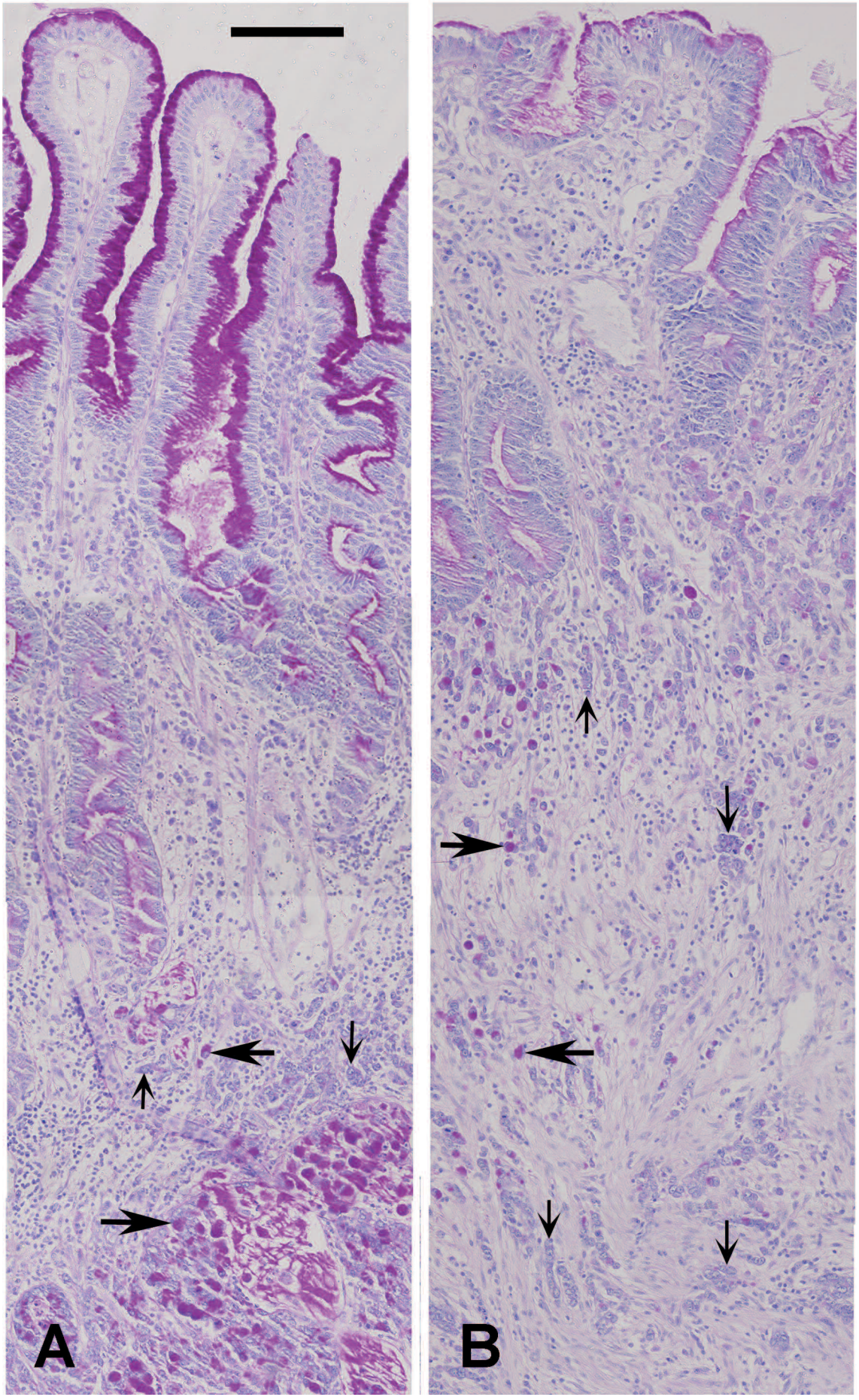

Fig. 4. Montages of light micrographs showing the gastric mucosae of the tumor edge **(A)** and tumor center **(B)** of the resected gastric cancer tissue. **A.** In the superficial portion of the mucosa of the "tumor edge", the gastric pits appear elongated, tortuous, and lined with cells producing much PAS-labeled mucus. The pits are continuous with short glandular profiles lined by cells producing little PAS-labeled mucus. Deep in the mucosa, note the presence of cancer cells which appear in two forms: large PAS-labeled mucus-producing cells (large arrows) and small poorly differentiated cells (small arrows). **B.** In the "tumor center" of resected gastric cancer tissue, the superficial portion of the mucosa shows gastric pits continuous with short glandular profiles lined by cells producing little PAS-labeled mucus. Note the presence of cancer cells deep in the mucosa (arrows). The PAS-labeled mucous producing cancer cells (large arrows) appear fewer than the small poorly differentiated cancer cells (small arrows). Bar: 50 μm.

absorptive cells with prominent brush borders which also stained positive for PAS (Fig. 3A). The surrounding stroma was packed with lymphoid cells. Two of these patients were females, the average age was 48 years, and interestingly, the three of them tested negative for *H. pylori* (Table 1).

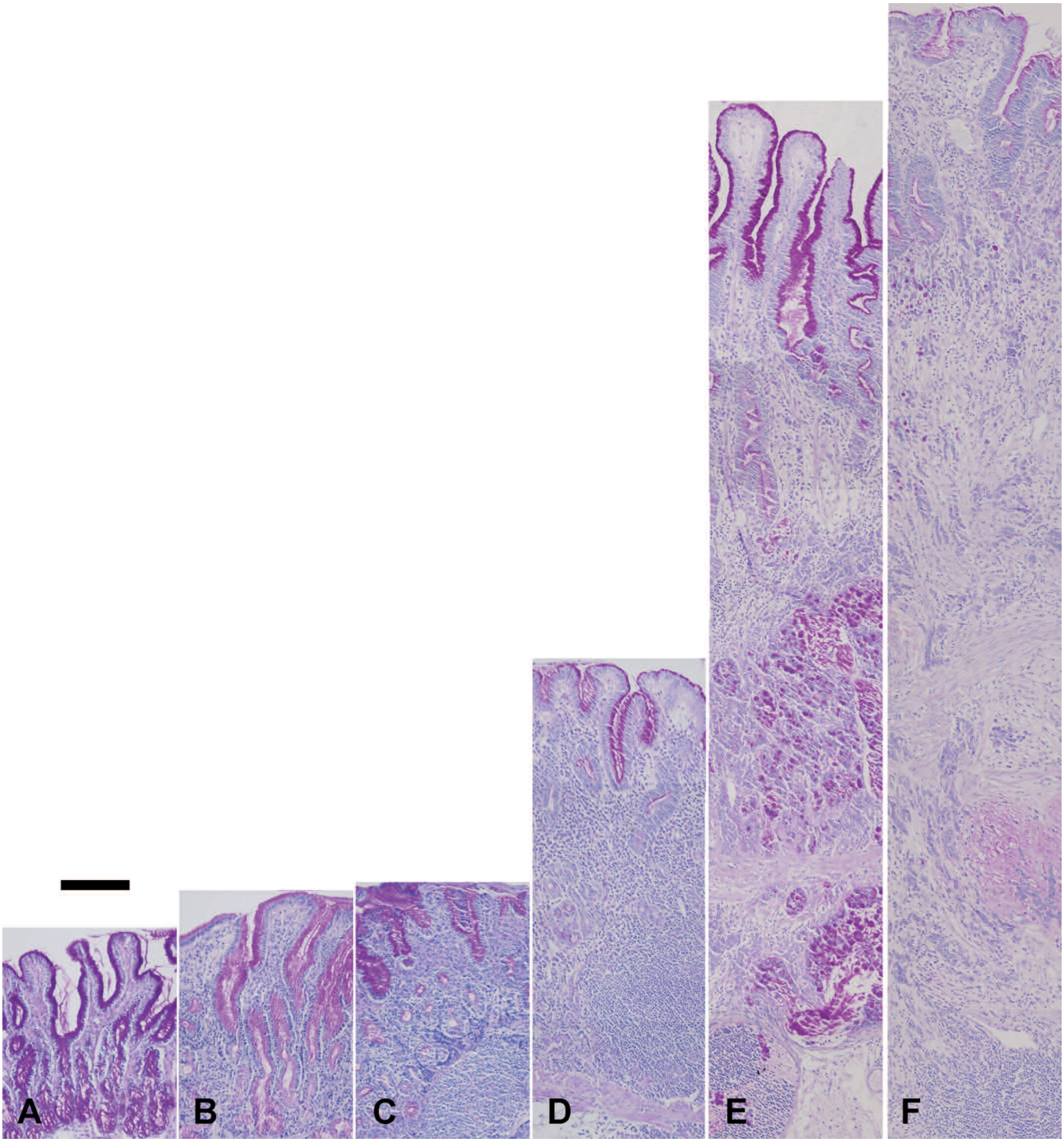

Fig. 5. Montages of images taken at the same magnification showing the differences in mucosal thickness and structure of normal antrum **(A)**, mild gastritis **(B)**, severe gastritis **(C)**, tumor safe margin **(D)**, tumor edge **(E)** and tumor center **(F).** Bar: 100 μm.

Morphological analysis of postoperative stomach tissues obtained from gastric cancer in the pyloric antrum

The age of the three pyloric antral cancer cases examined in this study averaged 55 years and two of them were males. *H. pylori* test was conducted only on one patient (male) and was negative (Table 1). Examination of mucosal tissues obtained from three different regions of the resected cancerous pyloric antral lesions revealed different structural features for each region.

a) Tissues obtained from the safe (resection) margin of the dissected part of the stomach showed morphological changes similar to, but more advanced than those described above in the case of severe gastritis. The thickness of the mucosa was obviously increased, mainly due to massive infiltration with lymphoid cells surrounding the ovoid epithelial profiles lined by progenitor-like cells with little mucus (Fig. 2B). The muscularis mucosa was also infiltrated by lymphoid cells. When all tissue blocks obtained from the safe (resection) margin of each gastric cancer patient were examined, it was possible to find several mucosal areas with evidence of intestinal metaplasia similar to the area presented in Fig. 3A, and dysplastic changes as shown in Fig. 3B. Dysplasia was in the form of dilated round or ovoid glandular profiles which appeared to lose the normal simple tubular appearance. The stroma around these dilated profiles included many lymphoid cells and the lumen was usually swamped with dead cells (Fig. 3B).

b) Tissues obtained from the tumor edge of the resected stomachs revealed variable changes. In some areas, the mucosa appeared more or less similar to that observed at the safe margin. However, the mucosal thickness was consistently increased. This was mostly due to infiltration of the mucosa with cancer cells forming round or ovoid epithelial profiles surrounded by massive amounts of lymphoid cells. In addition, changes observed in the surface epithelium and gastric pits contributed to the increased mucosal thickness. The surface epithelium was usually intact and made up of a monolayer of elongated mucous cells with invaginating gastric pits lined by similar mucous cells (Fig. 4A). The pits tended to appear very long and tortuous, extending deep into the mucosa. Pit cells were continuous with glandular cells which appeared to be immature, as indicated by the small amount of PAS-stained mucous granules in the apical cytoplasm. In some tissue blocks, deep in the mucosa, the glandular architecture was replaced by some invasive cancer cells. These infiltrating cancer cells formed aggregates deep in the mucosa and appeared in two forms: large round mucous cells (signet ring shape) and small immature cells (Fig. 4A). These cancer cells tended to mix and appeared in groups of variable shapes forming cords or clumps. These groups of malignant cells were also seen crossing the muscularis mucosa and invading the connective tissue and lymphatic or blood vessels in the submucosa (Fig. 5E).

c) Mucosal tissues obtained from the tumor center were variable in thickness. In some tissue blocks, the mucosal integrity was interrupted with discontinuous epithelium and signs of cell damage and necrotic connective tissue. In tissue blocks where the surface epithelium was intact, the mucosa appeared very thick (Fig. 4B). The glandular epithelia in some places deep in the mucosa were replaced by variable masses or groups of malignant cells (Figs. 4B, 5F). These cells appeared to be immature cells or were mucus-secreting with signet ring appearance as indicated by PAS labeling. When compared with the tumor edge, tissues obtained from the tumor center were infiltrated with more of the immature cancer cells and fewer mucous signet ring cells. In between these groups of cancer cells, connective tissue elements were rich in lymphoid cells which diffusely infiltrated the mucosa. In some tissue blocks, masses of malignant cells with signs of mucus production were captured during their invasion to the muscularis mucosa.

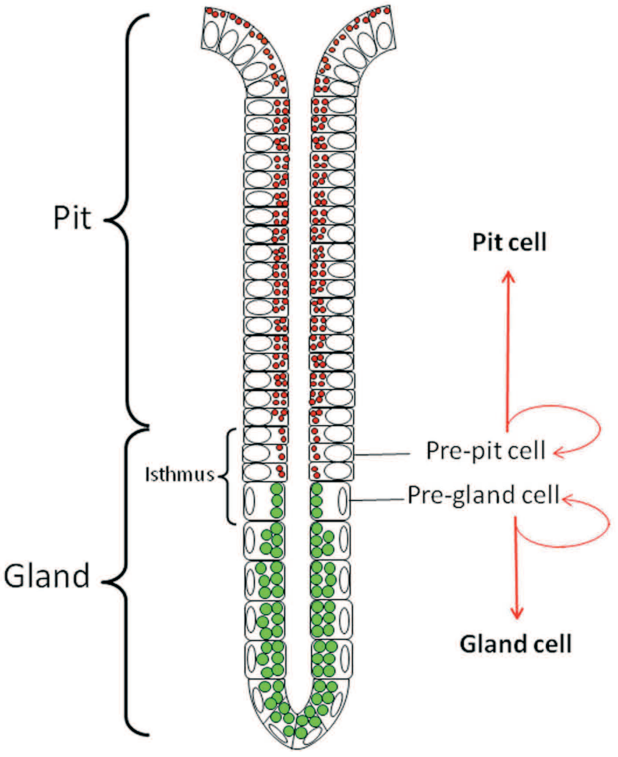

Fig. 6. Diagram representing the structure of the pit-gland unit in the antrum of the normal human stomach. The pit region is lined by pit mucous cells producing PAS-positive granules. The gland is lined by gland mucous cells producing large PAS-positive granules. Near the pit-gland border, there is a small region (isthmus) which contains few pit and gland mucous cells capable of mitosis. These cells divide to maintain themselves and to give rise to the post-mitotic pit and gland mucous cells. Therefore, these cells are taken to be the progenitors (pre-pit and pre-gland cells) of the pit-gland unit in the antrum of the human stomach.

Some groups of these mucus-secreting cancer cells were also seen within the connective tissue of the submucosa.

Discussion

The present study investigated the structure of the mucosa in microscopically normal human pyloric antrum, as well as the sequential changes that occur during development of gastritis and progression toward gastric cancer.

The structure of the pit-gland units in the pyloric antrum of normal human stomach

In mice, previous microscopic analysis of the stomach revealed that the most immature cell type in the pyloric antrum, and also the corpus region, is the granule-free cell located at the pit-gland junction or the isthmus region (Lee, 1985; Lee and Leblond, 1985; Karam and Leblond, 1992, 1993). Dynamic analysis using 3H-thymidine labeling and tracing of newly produced cells revealed that this immature cell type is not only capable of self renewal but also maturation-associated migration to maintain all gastric epithelial cell lineages (Lee and Leblond, 1985; Karam and Leblond, 1993). Because of their immature (embryonic cell-like) features and capacity of self renewal and production of all cell lineages, these granule-free cells were taken to be the pluripotent stem cell of the gastric epithelium (Lee and Leblond, 1985; Karam and Leblond, 1993).

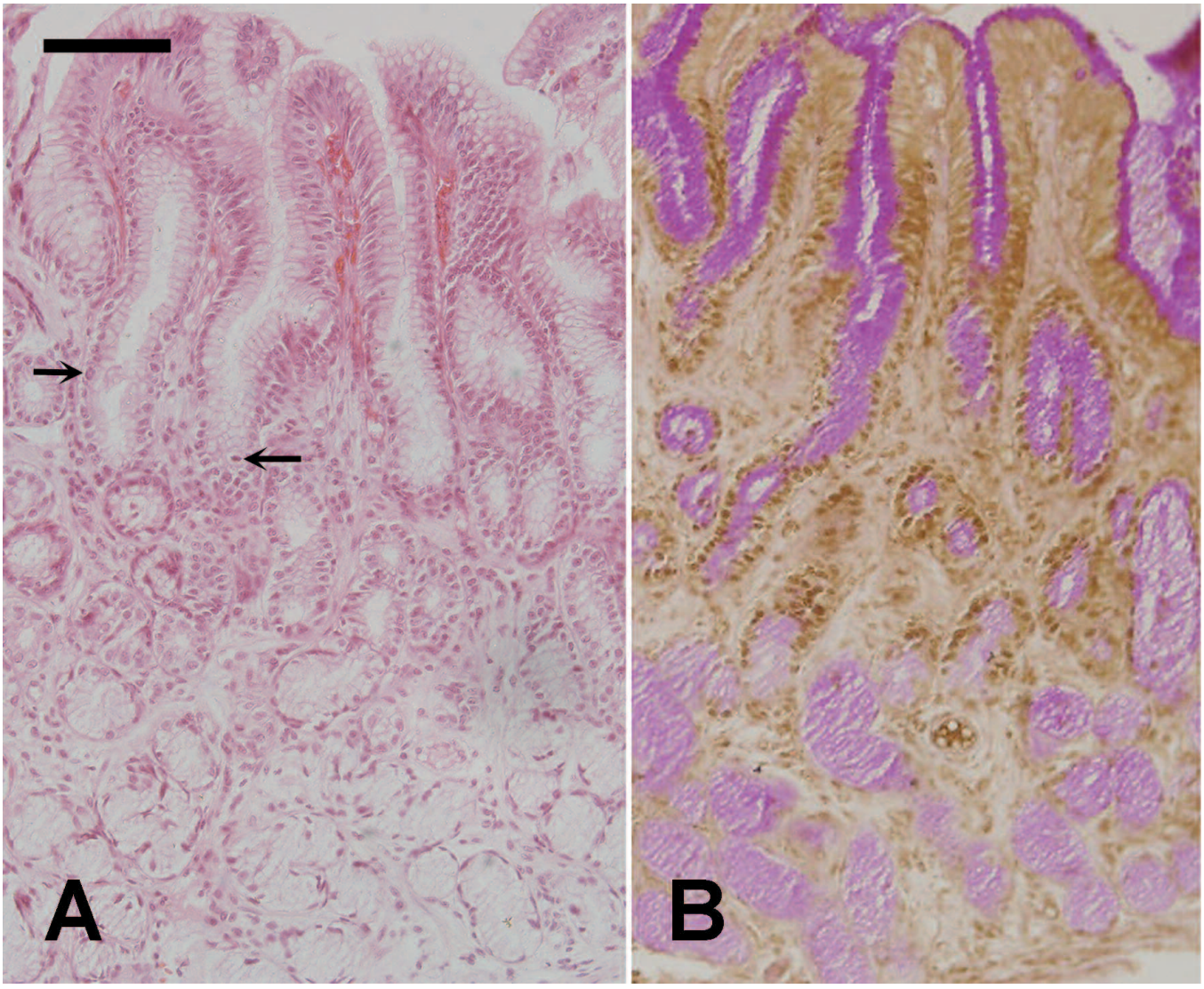

Fig. 7. Light micrographs showing adjacent tissue sections obtained from an antral biopsy with mild gastritis processed for hematoxylin and eosin staining **(A)** and immunohistochemistry using goat polyclonal anti-PCNA antibody **(B)**. While only a few mitotic figures (arrows) can be identified in **A**, many PCNA-labeled cells (brown nuclei) are seen in **B**. Bar: 40 μm

In humans, detailed electron microscopic analysis of the corpus region of the stomach revealed no such immature granule-free cells (Karam et al., 2003). The least mature cells were already committed or partially committed lineage progenitors called pre-parietal, pre-pit, pre-neck and mini-granule cells. These progenitors were all found at the pit-gland junction (isthmus region). Except for pre-parietal cells which had no secretory granules, but long apical microvilli, the cytoplasm of the three other progenitors contained a few small secretory granules (Karam et al., 2003).

In the present study, examination of the normal antrum of the human stomach revealed that the surface epithelium and the luminal surface of the whole lining of the pit and gland regions were positively stained with PAS (Fig. 1A). This indicates the presence of mucous granules in the apical cytoplasm of the lining epithelial cells, which include pit mucous cells and gland mucous cells. Therefore, except for the few scattered enteroendocrine cells, it seems that the whole surface lining of the epithelial units, including the pit-gland junction (isthmus), is composed of mucus-secreting cells (Fig. 1A).

Even though this study suggests that granule-free (stem) cells similar to those of the mouse stomach are not found in the epithelium lining the human pyloric antrum, evidence of self renewal is apparent. Mitotic figures are identified near the lower portion of the pit and in the upper portion of the gland (Fig. 1A, B). This portion of the gland is equivalent to the "isthmus" of the mouse pit-gland unit described previously by Leblond and colleagues (Lee, 1985; Lee and Leblond, 1985; Karam and Leblond, 1992, 1993; Karam et al., 2008). These mitotic cells tend to produce less mucus than mature (or differentiated) cells near the luminal surface or the gland bottom and, hence, are considered to be differentiating or progenitors. Briefly, in the pyloric antrum of the human stomach, differentiating mucus-secreting cells in the lower portion of the pit (or pre-pit cells) and differentiating mucous cells in the upper portion of the gland (or pre-gland cells) are capable of self renewal and also act as committed progenitors responsible for the production of mature pit and gland cells, respectively (Fig. 6).

The structural changes observed in pyloric antral biopsies with mild and severe chronic gastritis correlate with the updated Sydney system

In the present study, mucosal biopsies with inflammatory changes were classified into two main categories: mild gastritis and severe gastritis. Only a few

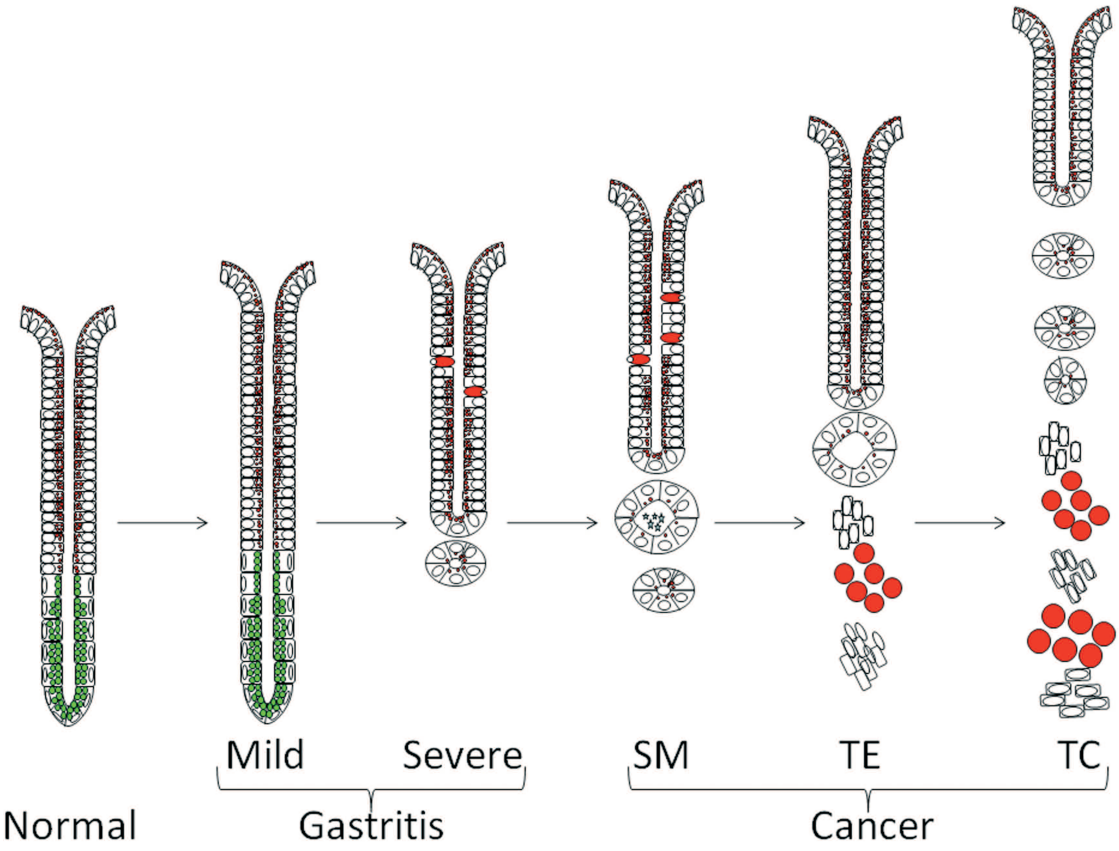

Fig. 8. Diagramatic representation of the changes that occur in pit-gland unit during the multistep process of gastric carcinogenesis in the pyloric antrum of the human stomach. Glandular atrophy and intestinal metaplasia which occur during gastritis are followed by dysplastic changes and eventually cancer cells develop and become invasive.

of the mild gastritis patients tested positive for *H. pylori* infection. However, many more patients tested positive for *H. pylori* in the severe gastritis group (Table 1). This is in line with the updated Sydney system which emphasizes the importance of etiology as one of the criteria to classify gastritis (Dixon et al., 1996; Stolte and Meining, 2001).

All patients involved in the present study were scheduled for endoscopic biopsy due to recurrent upper gastrointestinal symptoms, such as epigastric pain, heartburn, bloating, nausea and vomiting. The history and/or hospital record of the mild/severe gastritis patients showed *H. pylori* infection in some of them. It should be mentioned that no other etiological factor was reported, except that one of the mild gastritis and four of the severe gastritis patients were on non-steroidal anti-inflammatory drugs. Also, it should be noted that many of the mild (n=16) and severe (n=20) gastritis patients were on proton pump inhibitors or H2-receptor antagonists. It would be interesting to study the ultrastructural features of oxyntic mucosa in these patients. In normal rodents, we previously reported that inhibiting acid secretion by omeprazole or ranitidine was associated with alteration of parietal cells and enhancement of cell turnover in the oxyntic mucosa (Karam and Forte, 1994; Karam and Alexander, 2001). This could, at least partly, be attributed to hypergastrinemia associated with inhibition of acid secretion. Interestingly, when we used an antibody specific for the proliferating cell nuclear antigen (PCNA) to probe a tissue section of one of the mucosal biopsies diagnosed with mild gastritis from a patient who was receiving acid inhibitor, we noticed what appeared to be enhanced cell proliferation (Fig. 7). This issue requires further studies and analysis.

The structural changes of the gastric mucosa during chronic gastritis and cancer development support the multistep process of carcinogenesis

The tissue array of gastric mucosal biopsies and resected gastric cancer provided an opportunity to re-examine the multistep process of carcinogenesis which was originally proposed by Correa (1991). This process indicated that superficial gastritis is followed by chronic atrophic gastritis, which would lead to metaplastic and then dysplastic changes preceding the development of carcinoma (Correa, 1992; Correa et al., 2006).

In the present study, microscopic examination of the human antral biopsies made it possible to classify them into control (healthy), mild gastritis and severe gastritis (Table 1). The control tissues were not from normal volunteers, nor from gastric cancer patients (adjacent macroscopically normal tissue), but from patients with upper gastrointestinal symptoms referred for endoscopic biopsies and histological examination which revealed microscopically normal mucosa. Based on the morphological features of the available biopsies from each patient, some appeared healthy (n=25), others showed signs of mild gastritis (n=31), and the remaining showed evidence of severe chronic atrophic gastritis (n=33). A few of the latter were associated with intestinal metaplasia (n=3). Thus, the gastric epithelium was replaced with patches of precancerous lesions made of intestinal absorptive cells and scattered goblet cells, indicating the complete type of metaplasia (Correa et al., 2010).

Tissue sections of a representative biopsy for each of these three conditions (control, mild and severe gastritis) were assembled on a glass slide next to cancerous tissues from the three different regions: safe resected margin, tumor edge and tumor center. Interestingly, microscopic examination of these six assembled tissue sections appeared to form a series of progressive morphological changes (Figs. 5, 8). The number of lymphoid cells increased from biopsies with mild to severe gastritis. This was associated with atrophy of some antral glands. The gastric mucosae in the three tumor tissues showed a progressive increase in thickness from safe margin, to tumor edge toward the tumor center. The mucosa of the safe margin tissue was similar to that of chronic atrophic gastritis, but thicker due to the hyperplastic changes and increased ovoid epithelial profiles lined with immature mucous cells. In addition, the safe margin commonly showed signs of metaplastic and dysplastic changes. The tumor edge showed a mixed population of ovoid epithelial profiles made up of mucous progenitor cells and isolated immature cells and mucous signet ring cells. The later cell types were also involved in the local invasion of the muscularis mucosa and the submucosa. The tumor center appeared more advanced with a combination of epithelial profiles of progenitor cells in the superficial mucosa, along with large aggressive groups of immature and mucous cancer cells in the deep part of the mucosa. These cells were highly invasive and commonly seen inside blood vessels of muscularis externa. Therefore, the adenocarcinoma observed in the present study was a mixed intestinal-diffuse type which appears to have an increasing incidence (Henson et al., 2004).

Finally, the varied array of tissues studied appears to be representative and supportive of the multistep process of gastric carcinogenesis. This tissue array will be useful in testing the expression of important molecules such as potential tumor markers or factors involved in the early stages of gastric carcinogenesis.

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References

Brenner H., Rothenbacher D. and Arndt V. (2009). Epidemiology of stomach cancer. Methods Mol. Biol. 472, 467-477.

Correa P. (1991). The epidemiology of gastric cancer. World J. Surg. 15, 228-234.

Correa P. (1992). Human gastric carcinogenesis: a multistep and

multifactorial process--First American Cancer Society Award Lecture on Cancer Epidemiology and Prevention. Cancer Res. 52, 6735-6740.

Correa P., Piazuelo M.B. and Camargo M.C. (2006). Etiopathogenesis of gastric cancer. Scand. J. Surg. 95, 218-224.

Correa P., Piazuelo M.B. and Wilson K.T. (2010). Pathology of gastric intestinal metaplasia: clinical implications. Am. J. Gastroenterol. 105, 493-498.

Dixon M.F., Genta R.M., Yardley J.H. and Correa P. (1996). Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. Am. J. Surg. Pathol. 20, 1161-1181.

Ferlay J., Shin H.R., Bray F., Forman D., Mathers C. and Parkin D.M. (2010). Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int. J. Cancer 127, 2893-2917.

Fox J.G. and Wang T.C. (2007). Inflammation, atrophy, and gastric cancer. J. Clin. Invest. 117, 60-69.

Henson D.E., Dittus C., Younes M., Nguyen H. and Albores-Saavedra J. (2004). Differential trends in the intestinal and diffuse types of gastric carcinoma in the United States, 1973-2000: increase in the signet ring cell type. Arch. Pathol. Lab. Med. 128, 765-770.

Hohenberger P. and Gretschel S. (2003). Gastric cancer. Lancet 362, 305-315.

Kaminishi M. (2005). Diversity of gastric carcinogenesis. Oncology 69 (Supp 1),1-8.

Karam S.M. (2010). Mouse models demonstrating the role of stem/progenitor cells in gastric carcinogenesis. Front. Biosci. 15, 595-603.

Karam S.M. and Leblond C.P. (1992). Identifying and counting epithelial cell types in the "corpus" of the mouse stomach. Anat. Rec. 232, 231-246.

Karam S.M. and Leblond C.P. (1993). Dynamics of epithelial cells in the corpus of the mouse stomach. I. Identification of proliferative cell types and pinpointing of the stem cell. Anat. Rec. 236, 259-279.

Karam S.M. and Forte J.G. (1994). Inhibiting gastric H(+)-K(+)-ATPase activity by omeprazole promotes degeneration and production of parietal cells. Am. J. Physiol. 266 (4 Pt 1), G745-758.

Karam S.M. and Alexander G. (2001). Blocking of histamine H2 receptors enhances parietal cell degeneration in the mouse stomach. Histol. Histopathol. 16, 469-480.

Karam S.M., Straiton T., Hassan W.M. and Leblond C.P. (2003). Defining epithelial cell progenitors in the human oxyntic mucosa. Stem Cells 21, 322-336.

Karam S.M., Tomasetto C. and Rio M.C. (2008). Amplification and invasiveness of epithelial progenitors during gastric carcinogenesis in trefoil factor 1 knockout mice. Cell Prolif. 41, 923-935.

Lauren P. (1965). The two histological main types of gastric carcinoma: Diffuse and so-called intestinal-type carcinoma. An attempt at a histo-clinical classification. Acta Pathol. Microbiol. Scand. 64, 31-49.

Lee E.R. (1985). Dynamic histology of the antral epithelium in the mouse stomach: I. Architecture of antral units. Am. J. Anat. 172, 187-204.

Lee E.R. and Leblond C.P. (1985). Dynamic histology of the antral epithelium in the mouse stomach: II. Ultrastructure and renewal of isthmal cells. Am. J. Anat. 172, 205-224.

Miller S.J., Lavker R.M. and Sun T.T. (2005). Interpreting epithelial cancer biology in the context of stem cells: tumor properties and therapeutic implications. Biochim. Biophys. Acta 1756, 25-52.

Nomura S. and Kaminishi M. (2007). Surgical treatment of early gastric cancer. Dig. Surg. 24, 96-100.

Roder D.M. (2002). The epidemiology of gastric cancer. Gastric Cancer 5 Suppl 1, 5-11.

Senapati S., Sharma P., Bafna S., Roy H.K. and Batra S.K. (2008). The MUC gene family: their role in the diagnosis and prognosis of gastric cancer. Histol. Histopathol. 23, 1541-1552.

Smith M.G., Hold G.L., Tahara E. and El-Omar E.M. (2006). Cellular and molecular aspects of gastric cancer. World J. Gastroenterol. 12, 2979-2990.

Stolte M. and Meining A. (2001). The updated Sydney system: classification and grading of gastritis as the basis of diagnosis and treatment. Can. J. Gastroenterol. 15, 591-598.

Testino G. (2006). Gastric precancerous changes: carcinogenesis, clinical behaviour immunophenotype study and surveillance. Panminerva Med. 48, 109-118.

Testino G., Cornaggia M. and De Iaco F. (2002). Intestinal metaplasia, high-grade epithelial dysplasia and gastric cancer. Scand. J. Gastroenterol. 37, 1475-1476.

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