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Frequency of Atypical Mitosis in Intestinal Metaplasia of the Gastric Mucosa in Japanese Patients

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The morphologic characteristics of the mitotic figures present in intestinal metaplasia (IM) of the gastric mucosa were investigated in 70 concecutive gastrectomy specimens from Japanese nationals. The specimens contained, in addition, early gastric cancer of intestinal type (n=44) or of diffuse type (n=22). The remaining 4 were recorded as mixed. One hundred or more consecutive mitoses/specimens were studied by high-power microscopy in hematoxylin and eosin-stained preparations (at ×1000). A total of 7259 mitoses were recorded (mean 103.7 mitoses/case). Of these, 1089 mitoses (i.e. 19.1%) were considered as atypical according to a previous classification. The percentage of atypical mitoses was found to be unrelated to the gender, to the increasing age of the patients, to the histologic type of the adenocarcinoma contained in the specimens, or to the anatomic site (e.g. corpus or antrum or tumor proximity). Comparative studies were done with gastrectomy specimens from Swedish nationals (a population with a 4-times-lower incidence of gastric carcinoma than the Japanese). The results showed a much lower frequency of mitotic figures/specimen and only occasional atypical mitosis. Since atypical mitosis has so far been reported only for neoplastic lesions in the gastrointestinal tract, it is suggested that IM with atypical mitosis may be a genuine precancerous lesion in the gastric mucosa in Japanese subjects.

Key words: Atypical mitosis — Intestinal metaplasia — Gastric mucosa

Despite declining incidence in most parts of the world, gastric carcinoma remains the most common cancer form for the population at large.[1)] The causes of gastric carcinogenesis remain poorly understood.[2)] On the other hand, there is an increased awareness that certain histological changes in the gastric mucosa may predispose, antedate or concur with the development of gastric carcinoma. Those alterations are: chronic (atrophic) gastritis,[3)] intramucosal cysts,[4, 5)] dysplasia,[6)] adenomatos polyps[7)] and intestinal metaplasia.[8, 9)]

The possible relationship of intestinal metaplasia (IM) with gastric carcinogenesis is based on the following observations: a) IM increases with increasing age (a known high risk factor for cancer development), b) IM is often present in stomachs with gastric carcinomas, particularly those of intestinal histological type, c) IM is usually found in Japanese males, more often affected by gastric carcinomas than Japanese femals, d) IM often surrounds foci of minimal invasive gastric carcinoma (i.e. less than 5 mm in diameter), e) IM is more often found in countries with a high incidence of gastric carcinoma and f) IM is found in the gastric mucosa of rats carrying an experimentally induced highly differentiated adenocarcinoma.[10, 11)]

Previous studies indicate that Mexican nationals,[12)] a population with a low incidence of gastric carcinoma, have a low incidence of IM. On the other hand, Swedish nationals[13)] having a moderate incidence of gastric carcinoma demonstrate a moderate frequency of IM, while IM is frequently found in Japanese subjects,[13)] in whom the incidence of gastric carcinoma is high. More recently, we found by means of quantitative morphometry, a more extended IM in the gastric mucosa of the Japanese[14)] than in the gastric mucosa of the Swedes.[15)] The abovementioned data suggest that IM may be related to gastric carcinogenesis.

While studying the extension of IM in gastrectomy specimens from Japanese patients having early gastric carcinoma[16)] we noticed in most cases foci of IM with a relatively high frequency of dividing cells. The same phenomenon was seldom found in gastrectomy specimens from Swedish or Mexican nationals (preliminary results have been reported[17)]). The purpose of the present work was to study in more detail the morphologic characteristics of the dividing cells in a larger number of Japanese patients.

MATERIALS AND METHODS

From the files of the Department of Pathology, Cancer Institute, Tokyo, 82 consecutive gastrectomy specimens having early gastric cancer were analyzed. After removal, the specimens had been fixed in 10% neutral formalin for 3 days. After fixation, the specimens were cut into blocks measuring up to 4.5 cm in length as shown in Fig. 1. The specimens were stained in hematoxylin and eosin (H & E).

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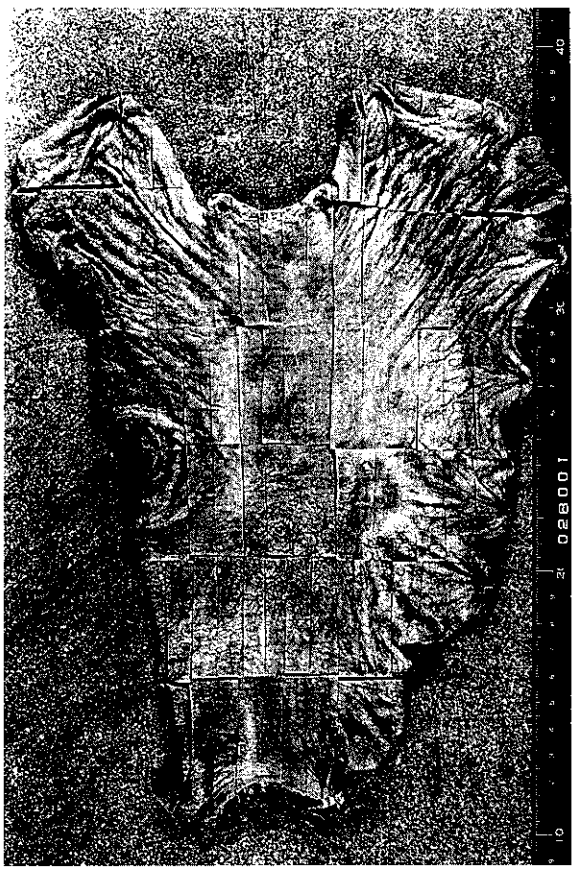

Fig. 1. Gastrectomy specimen showing total sectioning (into blocks measuring about 4.5 cm × 0.5 cm).

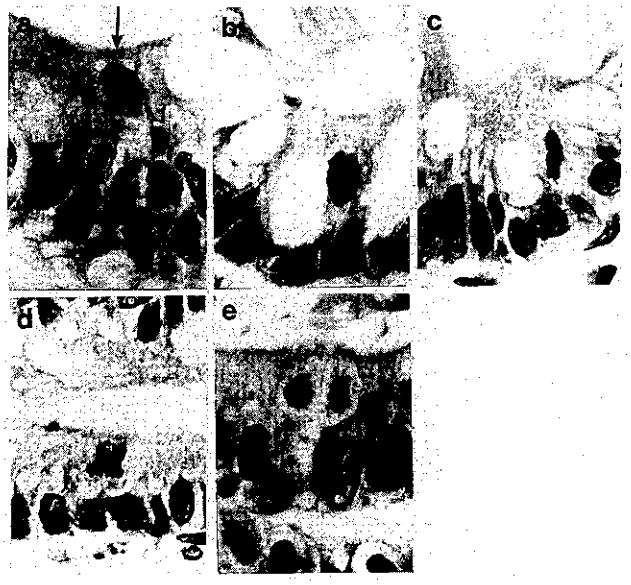

Fig. 2. Normal mitosis. a) Prophase: Fragmentation of the nuclear membrane with granular chromatine condensation (at arrow). b) Prometaphase: Formation of chromosomes. c) Metaphase: Chromosomal condensation in equatorial plate, in a vertical position. d) Early anaphase: Migration of chromosomes towards the cell poles. e) Telophase: Formation of two daughter cells (H & E, ×1000).

Areas with IM (without cellular atypias) were searched for the presence of mitotic figures. Seventy cases, having a total of 100 or more mitoses in 8 or less sections, qualified for the present investigation. All mitotic figures were classified into their various phases. Areas having "normal" gastric mucosa (without IM), usually with chronic inflammation, were also searched for mitotic figures. The sections (cut at 4 μm) were scrutinized under oil immersion (×1000). The mitotic figures were classified according to the criteria described below.

Normal mitosis (Fig. 2) 1) Normal prophase was characterized by fragmentation (i.e. loss of continuity) of the nuclear membrane with granular condensation of the nuclear chromatin. At the end of the prophase, the nucleus was poorly stained and the nucleoli could not be visualized. 2) Normal pro-metaphase. The chromosomes were now discernible and adopted a haphazard distribution in the nuclear area. 3) Normal metaphase. The condensed chromosomes were arranged along the equatorial plate of the cell. The position of the equatorial plate was vertical to the luminal-cytoplasmic interphase. 4) Normal anaphase. The migrating chromosomes were seen between the equatorial plate and the poles of the spindle. 5) Normal telophase was heralded by the reappearance of two distinct cells carrying nuclear membrane and one or more small, regular nucleoli.

Atypical mitosis (Fig. 3) 1) Atypical prophase was classified as such when the cell had a fragmented nuclear membrane, irregular clumps of chromatin, large nuclear diameter and/or one or more chromatin-free zone(s). 2) Atypical pro-metaphase was characterized by circular prometaphases with fringy nuclear boundaries, denoting irregular chromosomal arrangement, and/or misplaced segregated chromosomes (spill-overs). 3) Atypical metaphase. This group included metaphases showing irregularities in the equatorial plate with misplaced, segregated chromosomes, either at the centre or at the periphery of the equatorial plate. Other varieties of atypical metaphases were those adopting oblique or horizontal positions, semicircular or quasi-semicircular shapes and/or 3, 4 or more polar dividing cells. 4) Atypical anaphases were considered to be those with asymmetric migrating chromosomes lacking parallelity, with or without chromosomal bridging or segregated (lagging) chromosomes.

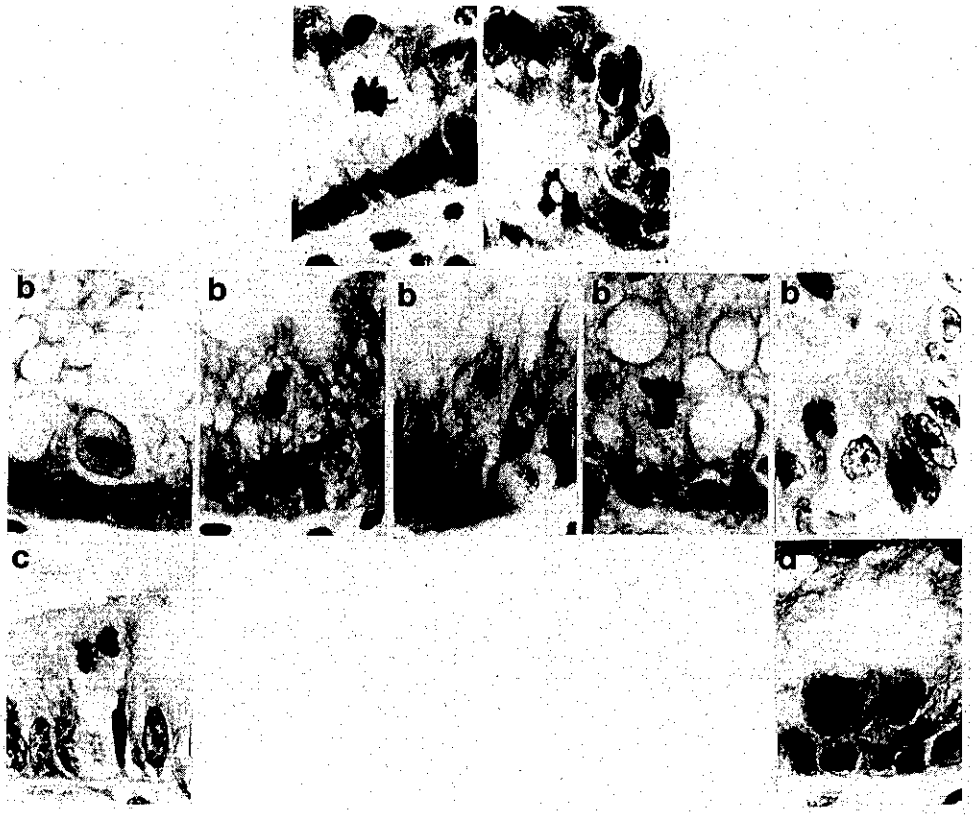

Fig. 3. Atypical mitosis. a) Samples of abnormal prometaphases: Circular arrangement of chromosomes with asymmetry and dispersion of chromosomes. b) Varieties of atypical metaphases: Horizontal position, angular shapes, lagging of chromosomes, tripolar and four polar mitoses. c) Varieties of atypical anaphases: Asymmetry of chromosomes and lack of parallelity with lateral dislocation. d) Telophase with asymmetry of daughter cells.

5) Atypical telophase included asymmetric daughter cells (nuclei) and/or nuclear irregularity with misplaced chromosomes.

RESULTS

Number of atypical mitoses in IM A total of 7259 mitoses were recorded in areas with IM in the 70 gastrectomy specimens (mean 103.7 mitoses/case, range 100–119 mitoses). Of the 7259 mitoses, 19.1% (i.e. 1389 mitoses) were considered as atypical. A mean of 19.8 atypical mitoses were recorded in each case (range 7–36).

Frequency of atypical mitoses in IM and gender Fifty-three of the 70 patients were male and the remaining 17, females. The mean percentage of atypical mitoses for males was 20.0 (SD 6.5, range 8–37) and for females, the mean was 19.05 (SD 7.2, range 7–30). No clear difference was found in the percentage of atypical mitoses between male and female patients.

The frequency of atypical mitoses in IM and age The results of this study are shown in Fig. 4. The mean percentage of atypical mitosis was 21.07 in patients $\leq$49 years of age; it decreased between 50 and 69 years of age and regained high values after 70 years of age. In fact, the highest mean percentage for atypical mitosis was recorded in this latter group. These results, however, do not substantiate the idea that the percentage of atypical mitosis increases (or decreases) with age.

The frequency of atypical mitoses in IM and the histologic type of the tumor In the present material, 44 of the 70 specimens had early gastric cancer of intestinal type, 22 had early gastric cancer of diffuse type and the remaining 4 were recorded as mixed. The results summarized in Fig. 5 indicate no essential differences between the two main groups (i.e. intestinal and diffuse type adenocarcinomas). The number of cases in the remaining group, namely "mixed intestinal and diffuse type adenocarcinomas" were too few to permit a comparison. However, the mean values of atypical mitosis for the 4 cases were similar to those for the rest of the meterial.

The frequency of atypical mitoses in IM and their topographic distribution The sections obtained for the study

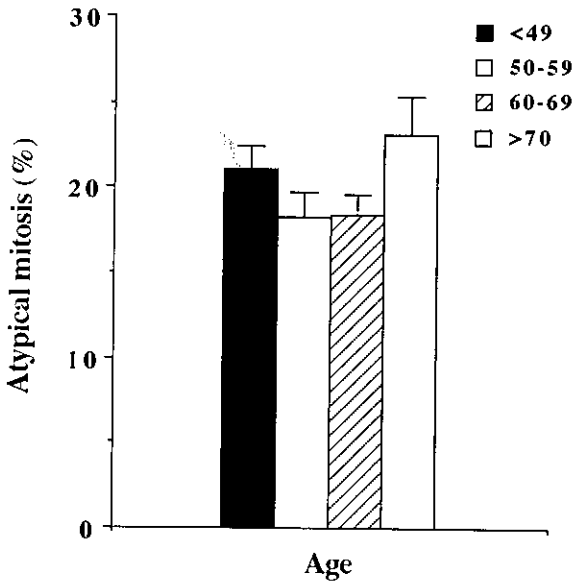

Fig. 4. The frequency of atypical mitosis in IM and age distribution in 70 consecutive gastrectomy specimens having early gastric cancer.

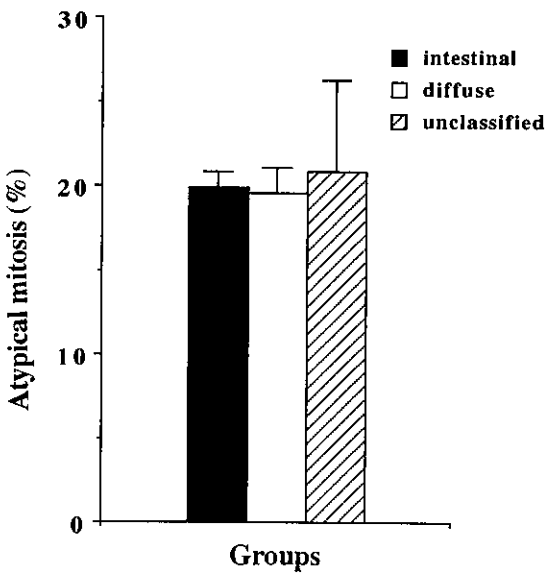

Fig. 5. The frequency of atypical mitosis in IM and the histological type of the tumor in 70 consecutive gastrectomy specimens having early gastric cancer of intestinal type (n=44), diffuse type (n=22) or mixed type (n=4).

of mitosis were from various regions of the stomach (i.e. corpus and antrum). The results demonstrated no trend concerning the presence of atypical mitosis in IM when the sections studied were either from the antrum or the corpus mucosa.

The frequency of atypical mitoses in the normal gastric mucosa (i.e. without IM) In only 2 of the 70 cases did normal gastric mucosa have ≥ 100 mitoses. One case had 1% atypical mitosis in areas with normal gastric mucosa (i.e. without IM) and the other, 2%.

DISCUSSION

The present investigation demonstrated that a large proportion of atypical mitoses may be present in areas of gastric IM in Japnanese patients having adenocarcinoma in the same gastrectomy specimen. The occurrence of atypical mitosis in IM has not been previously reported in the literature.

The percentage of atypical mitoses was higher in patients ≤ 49 years of age and in patients ≥ 70 years of age than in those patients between 50 and 69 years of age. While these findings are difficult to evaluate, it is apparent that the percentage of atypical mitosis does not increase (or decrease) with increasing age.

The percentage of atypical mitoses was found to be independent of the gender of the patients, of the histological type of the associated adenocarcinoma or of the number of sections required for the recording 100 mitoses/case.

It may be argued that the differences in the number of mitoses among the cases reviewed were due to differences in the time elapsed before surgical removal and fixation of the preparations.[18] However, early experiments at this Institute demonstrated that the mitotic frequency was similar at various time intervals (up to 7 h) before fixation,[19] irrespective of the temperature of the environment: some specimens were kept at 4°C and others at 25°C. Similarly, in a recent report on the mitotic rate in human soft tissues sarcomas, Donhuijsen *et al.*[18] found similar mitotic frequencies when the specimens were fixed up to 3 h following surgery. In the present work, all specimens were kept at 4°C and fixed within 3 h following surgical removal.

The occurrence of chromosomal anomalies in tumors from human subjects has been reported by cytogeneticists.[20] However, histopathologists have not applied such knowledge to the systematic analysis of the characteristics of the mitotic figures in mucosa adjacent to precancerous lesions or to invading neoplasms in human subjects. In studies of the characteristics of the mitotic figures in the gastrointestinal tract, we demonstrated that normal-looking mucosae (with or without inflammation) in areas adjacent to esophageal,[21] gastric[22] and colonic[23] dysplasias had a low proportion of atypical mitoses, i.e. 1.2%, 1.5% and 1.4%, respectively. Even

the duodenal mucosa of pediatric patients with celiac disease had a similarly low percentage of atypical mitosis (1.2%).[24] It should be stressed that the study on mitotic figures of the non-metaplastic gastric mucosa adjacent to a gastric adenoma[22] was based on the characteristics of only 20 mitoses (since the mitotic frequency in that mucosa was low). In the present work, only 2 of the 70 specimens showed 100 or more mitotic figures in the non-metaplastic gastric mucosa (with or without inflammation). The percentage of atypical mitoses in the latter 2 specimens was 1% and 2%, respectively. On the other hand, the percentage of atypical mitoses in areas with IM in those 2 specimens was 19% and 28%, respectively. From the results presented above, it may be inferred that the presence of atypical mitosis in the gastric mucosa of Japanese nationals with early gastric cancer may not be an overall phenomenon for that mucosa but related to areas with IM. Recently it was found[25] that complete IM in Japanese stomachs is present predominantly in the fundic mucosa, whereas incomplete IM is found in the mucosa of the antrum. The present results demonstrated no trend concerning the presence of atypical mitosis and their topographic distribution (whether in the fundic or in the antral mucosa). This was also the case for the frequency of atypical mitosis and tumor proximity.

In order to study whether atypical mitosis occurred in stomachs without adenocarcinoma, 10 consecutive gastrectomy specimens having peptic gastric ulcer (Japanese patients) were reviewed. Unfortunately, none of the 10 cases qualified for this study since only occasional mitoses (normal) were found.

In another investigation, this time in Swedish patients with early gastric cancer,[26] it was found that the mitotic frequency in IM was usually less than 100/case. In fact, only 2 of the 18 specimens contained ≥ 100 mitoses. The frequency of atypical mitosis in IM in those 2 specimens was 1.3 and 1.6, respectively (Rubio, unpublished). The number of mitotic figures in gastric IM among Mexican nationals is also low.[12]

Since atypical mitosis so far has been reported for neoplastic lesions of the gastrointestinal tract,[21–23] it is suggested that IM with atypical mitosis may be a genuine precancerous lesion in the gastric mucosa in Japanese subjects.

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