A Phenotypic Shift from Gastric-Intestinal to Solely Intestinal Cell Types in Intestinal Metaplasia in Rat Stomach Following Treatment with X-rays

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Abstract: Histological features and genetic changes induced by X-rays in intestinal metaplasia (IM) in rats were assessed by histochemistry and immunohistochemistry. A time course study and a PCR-SSCP analysis were performed. The IMs in rats were classified into two major types according to the cells forming the metaplastic glands. The first (the GI type) had both gastric and intestinal type cells forming the metaplastic glands. The second (the I-sol type) had solely intestinal cells forming the metaplastic glands. This characterization is similar to that used to define human IMs. The occurrence of IMs of the I-sol and GI types in rats gradually increased with time after X-ray irradiation. The number of IMs of the GI type was relatively high at 2 and 4 weeks after X-ray irradiation, and was low thereafter. On the other hand, the number of IMs of the I-sol type was extremely low at 2 weeks after treatment, then increased with time, and reached a maximum at 77 weeks after treatment. In the PCR-SSCP analysis, there were no alterations of the H-ras, K-ras, and p53 genes in the IM glands of rats treated with X-ray irradiation 8 weeks earlier. These observations suggest that the phenotypic change from IMs of the GI type to the I-sol type occurred without ras and p53 gene alterations.

Key words: rats, intestinal metaplasia, stomach, X-ray, phenotype

Introduction

Intestinal metaplasia (IM) is characterized by the phenotypic occurrence of intestinal cells in the gastric mucosa. Although IMs in humans are frequently observed in the gastric mucosa in cases of atrophic gastritis caused by infection of Helicobacter pylori, and have been associated with gastric cancer1–5, the actual relationship between IMs and malignancies is still unclear. Experimentally, IMs in rats have been reported to be induced by treatment with chemical carcinogens, physical stimulation or X-irradiation6–8. These results have been utilized in trying to predict the causes of human gastric cancer9, but the nature of IMs in humans and their relationship to the experimentally induced IMs in animals are still unknown. In particular, the fate of the induced IMs in rats and their morphological similarity to those in humans are not well established. Hence, to clarify the characteristics of the rat IM, we induced IMs in rats by treatment with X-ray irradiation, analyzed the changes after treatment and examined the phenotypes of the IMs using the same criteria used in the analysis of human IMs. In addition, to determine if any alterations in gene had occurred in the IM, PCR-SSCP methods were applied to the H-ras, K-ras, and p53 genes.

Materials and methods

Animals and X-ray irradiation

Male Hos:Donryu rats (6 weeks of age) were purchased from SLC Co., Ltd., Shizuoka, Japan. The animals were housed through the study period in polycarbonate cages (three or four animals per cage) bedded with hardwood chips, and were maintained in an air-conditioned room at constant temperature (24 ± 2°C) and relative humidity (50 ± 10%) with 12 hr light/dark cycles. They were given continuous access to commercial rat chow (Oriental MF, Oriental Yeast Co., Tokyo, Japan) and tap water. One hundred and nine rats received two doses of X-irradiation (10 Gy) with a 3 days interval between the doses, and the...
remaining 30 rats served as control animals. The dose of X-irradiation was determined in previous studies. For the X-ray treatment, the animals were anesthetized with Nembutal® (pentobarbital sodium) and placed in the X-ray beam. A 0.6 cm thick lead cover, with a hole 1.8 cm in diameter, was positioned so that the hole lay over the gastric region of the rats.

Histopathological examinations

To investigate the change in the number of IMs, the irradiated rats were sacrificed at 2, 4, 8, 24, 40, and 77 weeks after X-irradiation, under deep anesthesia with ether. The untreated, control rats were also sacrificed at 8 and 40 weeks after irradiation. All the animals were administered BrdU (50 mg/kg body weight) intraperitoneally 1 hr before each autopsy to identify the proliferating cells. The stomach was removed from each rat, opened along the large curvature, placed serosal side down on a piece of cardboard, and then fixed in 10% neutral buffered formalin solution. Eight strips including the pyloric and fundic mucosa were cut and embedded in paraffin. Forty eight serial sections of 5 µm thickness were obtained from each paraffin block and divided into 6 sets of 8 continuous sections. Each set was stained in order by hematoxylin-eosin, Alcian-blue (pH 2.5) and periodic acid Schiff reaction double stain (AB/PAS), paradoxical concanavalin-A (type III mucin) and immunohistochemical tests for BrdU, intestinal alkaline phosphatase (I-ALP) and cathepsin E were performed. The characteristics of the IM were identified by the existence of intestinal phenotypes, defined by the occurrence of goblet metaplasia and positive I-ALP reactivity in the immunohistochemistry, and gastric phenotypes, defined by positive type III mucin and negative cathepsin E immunohistochemistry. The intestinal metaplasias were classified according to the classification system reported for humans.

Gene alterations

A further 15 rats were treated with X-irradiation using the same methods to those described above. Eight weeks afterwards, these rats were used for the preparation of DNA samples. Sections of 5 µm thickness taken from paraffin embedded gastric tissues were stained with hematoxylin and the metaplastic gland of the mucosa was microdissected under low magnitude microscopy, using disposable needles. Two to six DNA samples from each treated rat were extracted for studying each gene using DEXPAT (Takara Shuzo, Ohtsu, Shiga), following the manufacturer’s instructions. For assessment of the genetic changes, K-ras exons 1 and 2, H-ras exons 1 and 2, and p53 exons 5 to 9 were selected. Mutations were screened by the polymerase chain reaction (PCR) - single strand conformational polymorphism (SSCP) method, as described previously. The primers used are listed in Table 1.

Results

Histological findings

There were no neoplastic changes in the rat gastric mucosa at any time after X-irradiation. In the rats sacrificed 2 weeks and 4 weeks after X-irradiation, partial regenerative changes were observed in the gastric mucosa. The regenerative changes were not observed in rats sacrificed at 8 weeks after X-ray treatment, or later.

Type of intestinal metaplasias

The metaplastic glands were classified into two major types, based on the cell type forming the glands. One (the GI type) consisted of a mixture of both intestinal and gastric cell types, and the other (the I-sol type) consisted solely of intestinal type cells. The GI and I-sol subtypes of IM were identified according to their cell compositions, which were distinguished by their phenotypes. These were, in turn, identified by histochemistry or immunohistochemistry. The

| Table 1. Primers Used for PCR-SSCP Analysis of Intestinal Metaplasia of Rats Treated with X-irradiation |
|-----------------|-----------------|-----------------|-----------------|
| Gene | Exon | Sequence | Name |}
| p53  | 5    | 5'-GAT TCT TTC TCC TCT CCT AC-3' | R5U  |
|      | 5    | 5'-ACA GCC AGT GCC AGT GVT CA-3' | R5D  |
|      | 6–7  | 5'-CCT CTG ACT TAT TCT TGC TC-3' | R6U  |
|      | 5    | 5'-AAC CTG GCA CAC AGC TTC CT-3' | R7D  |
|      | 8–9  | 5'-CTT GTG CTG TGC CTC CTC TT-3' | R8U  |
|      | 5    | 5'-CCA ATA ATA ACC TGG GTA CC-3' | R9D  |
| Ha-ras | 1    | 5'-ATG ACA GAA TAC AAG TTT CT-3' | RHA1U |
|      | 5    | 5'-GGC AGG TAG TCA GAG CTC AC-3' | RHA1D |
|      | 2    | 5'-AGG ACC TCT TAA CAG GTG GTC CT-3' | RHA2U |
| Ki-ras | 1    | 5'-ATG ACT GAG TAT AAA CCTT TT-3' | RKL1U |
|      | 5    | 5'-AGC AGC ATT TAC CTC TAT CG-3' | RKL1D |
|      | 2    | 5'-CAG GAC TCC TAC AGG AAA CA-3' | RKL2U |
|      | 5    | 5'-GAT TTA GTA TTA TTT ATG GC-3' | RKL2D |
IM GI type is characterized by the presence of intestinal phenotypes, such as I-ALP positive cells and/or goblet cells, in the metaplastic glands of the fundic or pyloric mucosa (Figs. 1, and 2). The IM I-sol type consisted of small intestinal cells of the absorptive and goblet type, and sometimes contained Paneth cells (Fig. 3). The absorptive type cell has a well-developed striated cell border with I-ALP reactivity, and the goblet type cell has large secretory granules that are stained by AB (pH 1).

The metaplastic glands exist in both fundic and pyloric mucosa as single metaplastic glands (single IM) or aggregates of metaplastic glands (multiple IM) of a few in number. In the latter case, the glands forming the aggregates were mostly either the I-sol type or both the GI and I-sol type, and were rarely only GI type glands (Fig. 4).

**Incidence of metaplastic glands**

The total number of IMs per fixed distance of fundic and pyloric mucosa increased with time after irradiation (Table 2, Figs. 5, and 6). At 2 weeks after irradiation, the majority of IMs were the GI type. The occurrence of the GI type of IM reached a maximum at 4 weeks after irradiation, decreased at 8 and 40 weeks after irradiation, and slightly increased again at 77 weeks after irradiation. The I-sol type of IM was rarely observed at 2 weeks after irradiation, but this type of IM in fundic mucosa increased at 24 weeks after irradiation and remained almost constant at 40 and 77 weeks after irradiation. In pyloric mucosa, the I-sol type of IM gradually increased with time and reached a maximum at 77 weeks after irradiation.

The proportion of metaplastic glands consisting of a single IM and multiple IMs in the fundic and pyloric mucosa changed with time after irradiation (Figs. 7, and 8). At 2

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**Fig. 1.** Focus of metaplastic glands in the fundic mucosa of rats sacrificed at 8 weeks after X-irradiation. A: Alcian-blue (pH 2.5) / PAS, B: I-ALP immunohistochemistry, C: cathepsin E immunohistochemistry, and D: paradoxical concanavalin-A staining. Normal glands (left side) and metaplastic glands are shown. Reactivity to cathepsin E is found in chief cells and mucosa neck cells of the normal glands and in some cells of the metaplastic glands. Characteristics of both gastric (type III mucin) and intestinal (goblet cells) are present in the GI type metaplastic gland. (Bar shows 50 µm).
Fig. 2. Metaplastic glands of the GI type in the pyloric mucosa of rats sacrificed at 8 weeks after X-irradiation. A: HE staining, B: Alcian-blue (pH 2.5) / PAS staining, C: I-ALP immunohistochemistry, and D: paradoxical concanavalin-A (type III mucin) staining. Two metaplastic glands are shown. The left gland shows both goblet cells and pyloric gland cells containing type III mucin. (Bar shows 50 µm).
Fig. 3. Metaplastic glands of the I-sol type in the pyloric mucosa of rats sacrificed at 8 weeks after X-irradiation. A: HE staining, B: Alcian-blue (pH 2.5) / PAS staining, C: I-ALP immunohistochemistry, and D: Cathepsin E immunohistochemistry. The cells of the metaplastic gland have well-developed striated cell borders with I-ALP reactivity and have no reactivity against cathepsin E. Allows show Paneth cells. (Bar shows 50 µm).
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Fig. 4. Focus of metaplastic glands at 24 weeks. A: Alcian-blue (pH 2.5) / PAS, B: I-ALP immunohistochemistry, C: cathepsin E immunohistochemistry, and D: paradoxical concanavalin-A staining. Five to six metaplastic glands are aggregated in the pyloric mucosa of rats sacrificed at 24 weeks after X-irradiation. A GI type IM is sandwiched between the I-sol type IMs. (Bar shows 100 µm).

Fig. 5. Change in incidences of I-sol and GI type IMs in fundic mucosa of the rats treated with X-irradiation.

Fig. 6. Change in incidence of I-sol and GI type IMs in pyloric mucosa of the rats treated with X-irradiation.
weeks after irradiation, almost all the IM glands consisted of a single IM, and the majority of these were IMs of the GI type. The ratio of metaplastic glands consisting of multiple IMs to those consisting of a single IM increased with time and reached a maximum at 77 weeks after irradiation. This tendency was particularly evident in fundic mucosa. At 77 weeks after irradiation, the most common type of metaplastic gland in the fundic and pyloric mucosa was the I-sol type gland consisting of multiple IMs.

**PCR-SSCP analysis**

There were no alterations in the PCR-SSCP analysis of metaplastic glands from the pyloric mucosa of X-irradiated rats, compared with the equivalent analysis of normal gastric glands in control rats that had not been irradiated.

**Discussion**

In this study, there were no adenomatous hyperplasias or adenocarcinomas of the gastric mucosa observed throughout the 77 weeks observation period, following X-irradiation. In addition, alterations of genes which have been reported to be related to gastric carcinogenesis in human and rats could not be detected by PCR-SSCP analysis in the IMs of rats sacrificed 8 weeks after X-ray treatment. These results suggest that the X-irradiation used in this study is sufficient to induce an intestinal metaplasia (IM), but not sufficient to induce a neoplasm in the gastric mucosa.

The IM induced by X-irradiation could be divided into major two types, those (the GI type) consisting of a mixture of gastric and intestinal cells and those (the I-sol type) consisting of solely intestinal cells, as previously reported in...
humans. The GI type IMs and some of the I-sol type IMs are equivalent to incomplete intestinal metaplasia, as proposed by other workers. Some subtypes of the GI type have been noted in metaplastic tubules in the gastric mucosa in humans, but the exact subtypes in rats could not be determined because of the small size of the rat gastric pits. This makes it difficult for many types of phenotypic cells to occur in a single gland, in contrast to humans. The classification used in this study showed that the IMs of rats are similar to those of humans in that the metaplastic glands contain similar cell phenotypes. This suggests that experimentally induced IMs in rats are useful tools for studying the pathogenesis of human IMs.

The number of IMs at 2 or 4 weeks after treatment was relatively high. This may be related to regenerative changes in gastric mucosa induced by X-irradiation, because the occurrence of IMs at 8 weeks after treatment decreased with disappearance of the regenerative changes. On the other hand, a gradual increase in the number of IMs at 24, 40, and 77 weeks after treatment reflects both the generation and the multiplication of IMs (Figs. 7, and 8). Although the mechanisms of multiplication are not clear from this study, there is no evidence of metaplastic tubules dividing into two or more daughter metaplastic tubules. Hence, this may indicate that the normal glands surrounding the IM are changing from a gastric to a small intestinal phenotype. This suggestion is supported by our observation that the number of multiple IMs of the GI type, which are regarded as glands undergoing a change from a gastric to an intestinal phenotype, increased at 77 weeks after treatment in the fundic mucosa. These results suggest that the generation of an IM may be affected by the surrounding microenvironment, such as the mesenchymal condition, the luminal acidity and/or the neighboring glands. This suggestion is also compatible with a report of the appearance of an IM adjacent to a primary gastric lymphoma in humans. It has been reported that an IM may be eliminated by Helicobacter pylori eradication, and, based on previous observations, IM formation is also considered to be a reversible change affected by the acidity of the gastric juice.

During embryonic development, the intestinal and stomach epithelium develop from the visceral endoderm. An in vitro investigation of growth factors and substrata in the development of the forestomach, glandular stomach and duodenal epithelial cells of rats showed that the glandular stomach epithelial cells exhibited intermediate characteristics between the forestomach and the duodenal epithelial cells, in terms of their growth factor requirements and substratum dependency. In addition, an analysis using co-culture and tissue grafting of the fibroblast lines of the fetal gut endoderm suggested that the fibroblasts modified the function, organization and/or gene expression of the overlying intestinal epithelium. These reports also support our suggestion that the mixed gastric and intestinal type intestinal metaplasia is a gland undergoing a change from a gastric to an intestinal phenotype, without ras and p53 gene alterations.

Recent work has also shown that intestinal development is associated with CdX1 and Cdx2 expression. In this study, we did not determine whether these homeobox genes affect the IM formation and the phenotypic changes. Hence, the details of the pathogenesis of the rat gastric intestinal metaplasia remain as an important problem to be solved.

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References


