Mini Review

Preneoplastic Changes of Stomach Cancer
—Mice, Rats and Mongolian Gerbils—

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Abstract: Investigations of the clonal growth of gastric carcinomas clearly suggest that individual cancers are derived from single cells with multi-potential activities and that cellular differentiation of gastric cancer cells occurs secondarily. By mucin histochemistry, gastric cancer cells of each histological group could be clearly classified into a gastric type and an intestinal type. The present results suggest the independent induction of intestinal metaplasia and gastric cancers and the occurrence of change of phenotypic expression of tumor cells from the gastric type to the intestinal type during growth of tumors. The Pepsinogen altered pyloric gland (PAPG) detected immunohistochemically may be considered to be a preneoplastic change. Rare mutations of p53 and ras genes in rat and mouse stomach cancers were found and p53 knockout mice (-/-) are more susceptible than (+/-) or (+/+) mice to N-methyl-N-nitrosourea stomach carcinogenesis. (J Toxicol Pathol 2002; 15: 133–136)

Key words: preneoplastic changes, stomach cancer, intestinal metaplasia, phenotypic expression of tumor cells, animals

Introduction

The phenotypic expression of tumor cells is widely thought to resemble that of the tissue of origin of the tumor cells. Thus, examination of phenotypic expression of stomach cancers should in theory reveal their histogenesis and for this reason intestinal metaplasia has been considered to be a possible precancerous state. However, we have proposed the independent induction of intestinal metaplasia and gastric cancers in rats¹. To investigate the cellular origin of tumors, the clonal growth of gastric carcinomas, cellular differentiation of rat glandular stomach cancers and mutations of tumor related genes were investigated.

Clonal Growth of Gastric Cancer

To investigate the clonality and cellular origin of gastric preneoplastic and neoplastic lesions, C3H/HeN→BALB/c chimeric mice treated with MNU were investigated immunohistochemically using a specific antibody to C3H strain specific antigen (CSA) enabling immunohistochemical discrimination of C3H cells in histological sections of chimeric mouse tissues. In normal gastric mucosa of the chimeras, each gland was composed entirely of CSA positive or negative cells and no mixed glands were found, indicating that each gland in the adult mouse is derived from a single progenitor cell. It follows that the surface mucous cells and pyloric gland cells in a pyloric gland are descended from multipotent single progenitor cells in each case. Cells of adenocarcinomas in chimeric mice treated with MNU were similarly homogeneous for one or other of the parental types, while comprising both surface mucous cell and pyloric gland cell forms. In addition, polyclonal tumors arose secondarily during progression, after two or more lesions coalescing. The results clearly suggest that individual cancers are derived from single cells with multi-potential activities and that cellular differentiation of gastric cancer cells occurs secondarily².

Cellular Differentiation and Histogenesis of Rat Glandular Stomach Cancers

The gastric and intestinal phenotypic expressions of tumor cells in adenocarcinomas induced by N-methyl-N‘-nitro-N-nitrosoguanidine (MNNG) or 4-nitroquinoline-1-oxide in the rat glandular stomach were studied by histochemical stainings for mucin and immunohistochemical staining for pepsinogen isozyme 1 (Pg1). By histochemical staining for mucin [by the paradoxical concanavalin A method, the modified method with labeled peanut lectin, the galactose oxidase-Schiff (GOS) reaction, and the sialidase-GOS reaction] and immunohistochemical staining of Pg 1, gastric cancer cells of each histological group could be
clearly classified into a gastric epithelial cell type, including pyloric gland cell, and surface mucous cell subtypes, and an intestinal epithelial cell type, including goblet-cell, and intestinal absorptive cell subtypes (Fig. 1). All tumors examined in this work consisted mainly of gastric-type cells but intestinal-type tumor cells were occasionally found among the gastric-type tumor cells. The incidences of intestinal-type cells in adenomas (11.1%) and small well-differentiated adenocarcinomas (28.6%) were significantly less (P<0.05) than that in large well-differentiated adenocarcinomas (68.4%). The incidence of intestinal-type cells in small undifferentiated adenocarcinomas (25.0%) was also less than that in large ones (58.3%). The present results suggest the occurrence of change of phenotypic expression of tumor cells from the gastric type to the intestinal type during growth of tumors3.

Independent Induction of Intestinal Metaplasia and Gastric Cancer in Rats

Intestinal metaplasia in pyloric mucosa and the presence of intestinal epithelial cell type tumor cells in gastric tumors in Wistar rats treated with MNNG were examined. Changes in the pyloric mucosa associated with MNNG treatment included intestinal metaplasia, adenomas and well-differentiated adenocarcinomas. Cells in pyloric mucosa of all rats and in both adenoma and well-differentiated adenocarcinomas (MNNG-treated rats could be classified into two categories: Gastric epithelial cell(G) type (pyloric gland cell type and surface mucous cell type) and Intestinal epithelial cell(I) type (intestinal-absorptive cell type and goblet-cell type). Intestinal metaplasia appeared in the pylorus, first in MNNG-treated rats 16 weeks after the beginning of MNNG administration, and later in untreated control rats after 40 weeks. The percentage areas of intestinal metaplasia per area of pyloric mucosa were always less than 1%. Adenomas without I type cells appeared earlier than did adenomas with I type cells. Only cells of G type were observed in 21 of 30 adenomas and in 19 of 36 well-differentiated adenocarcinomas. The others consisted chiefly of cells of the G type but also contained cells of I type. However, the percentage area occupied by I type was extremely small, being less than 3.5%. The present results showed gastric adenocarcinomas induced by MNNG in rats to be composed mainly of tumor cells of G type. These findings also suggest that neoplastic germ cells produce mainly G-type cells and only sometimes differentiate to I-type phenotype. Most tumors apparently originate from areas of mucosa composed only of G-type cells (Fig. 2).

Pepsinogen Altered Pyloric Gland (PAPG) as a Biomarker on Stomach Carcinogenesis

We have concentrated attention on Pg1 as a marker of preneoplasia. Pepsinogen isozymes (Pg) 1–4 have been isolated from the normal rat grandual stomach, three (Pg1, 3,4) occurring in the pyloric mucosa, and the four (Pg1-4) in the fundic glands. Pg1 expression preferentially decreases or disappears in pyloric mucosa during the early stages of rat MNNG-induced gastric carcinogenesis before morphologically distinct preneoplastic changes become evident5,6. Immunohistochemically, the alteration of Pg1 expression can be readily detected as pepsinogen 1 altered pyloric glands (PAPG) in normal appearing pyloric mucosa, and also consistently in adenomatous hyperplasias and adenocarcinoma consistently. Induction of PAPG was found to be dependent on the dose of MNNG administered and their numbers increase with time, the PAPG cells having a higher proliferation activity than their normal counterparts. In addition, the susceptibility of rats and mice to induction of gastric carcinoma by MNNG or MNU correlated with the susceptibility to induction of PAPG in each strain. Thus PAPG are now generally accepted as preneoplastic changes in the glandular stomach of rats and mice.

Reversibility of Tumor-like Proliferative Lesions in Glandular Stomach of Helicobacter pylori-Infected Mongolian Gerbils on Eradication

In 1994, the World Health Organization/International Agency for Research on Cancer concluded that “Hp is a
definite carcinogen” based on the epidemiological evidence. For detailed analysis of the role of Hp in stomach carcinogenesis, it is essential to establish a small animal model. We have established experimental models of stomach carcinogenesis in Mongolian gerbils (MGs) using the chemical carcinogens, MNNG and MNU. Hp infection enhances glandular stomach carcinogenesis in MGs treated with MNNG or MNU. And Hp infection exerts stronger promoting effects than a high-salt diet on gastric carcinogenesis, and that the two factors act as synergistically to enhance development of stomach cancer. Eradication diminishes enhancing effects of Hp infection on glandular stomach carcinogenesis in MGs.

Two papers have described well-differentiated adenocarcinomas induced in MGs’ stomachs by Hp infection without any exogenous chemical carcinogen. In our work, however, no gastric carcinomas were found in 15 MGs after two years of Hp infection. Tumor-like proliferative lesions (TLPs) also frequently develop with Hp infection in the glandular stomach of infected gerbils, with no obvious associated dysplastic changes of constituent cells. Distinguishing reversible inflammatory lesions from true neoplasms upon eradication is necessary for further biological or histochemical investigations using this model. We verified an experimental model of long-term Hp infection and eradication in gerbils. TLPs finally developed with a phenotypic shift of intestinalization with Paneth cells. After eradication, TLPs were obviously reduced, and gastric lesions in mucosa also improved with few remnants of former injury. This fact proved the existence of reversible TLPs frequently induced solely with Hp infection in this animal species, related to severe gastritis, rather than malignant in character. Thus, The role of Hp infection thus appears to be strong promotional influence, rather than initiation of gastric carcinogenesis.

**Rare Mutations of p53 and ras Genes in Rat and Mouse Stomach Cancers**

Rat: we examined the genetic alterations reported in human stomach cancers in 10 rat stomach cancers that had been induced in male ACI/N rats by administering MNNG in the drinking water. One of the 10 cancers had a mutation of the p53 gene at the second position of codon 171 (Val Glu). However, none of the 10 cancers had mutations in codons 12, 13, or 61 of Ki-ras or in the N-terminal phosphorylation sites of the beta-catenin gene. Southern blot analysis showed no amplification of K-sam or c-erbB-2 in the seven cancers examined. Finally, we searched for microsatellite alterations in 12 loci in nine cancers, but no alterations were observed. As these genetic alterations are observed in only a minor fraction of human stomach cancers, further analysis of genetic and epigenetic alterations in MNNG-induced rat stomach cancers is needed to disclose the major mechanisms of stomach carcinogenesis.

Mouse: The incidence of point mutations of H-, K- and N-ras and p53 oncogenes in male BALB/c mouse stomach tumors induced with MNU was examined by direct sequencing and PCR single-strand conformation polymorphism (PCR-SSCP). A mutation of GGT to AGT at K-ras codon 12 was found by SSCP in one adenocarcinoma from a total of 19 specimens including 5 adenocarcinomas, 9 adenomas, 1 squamous cell carcinoma and 4 normal-like stomach regions from 4 mice. No mutations were detected by direct sequencing of H-, K- and N-ras oncogenes at exons 1 (codons 12 and 13) and 2 (codon 61) in a total of 26 specimens comprising 10 adenocarcinomas, 10 adenomas, 2 squamous cell carcinomas and 4 normal-like stomach regions from 6 mice. No mutations were detected by direct sequencing of p53 oncogene at exons 5, 6, 7 and 8 in a total of 30 specimens including 13 adenocarcinomas, 8 adenomatous hyperplastic regions, 2 squamous cell carcinomas, 1 papilloma and 6 normal-like stomach regions from 7 mice. These results suggest that ras and p53 oncogenes do not play a role in mouse stomach carcinogenesis induced by MNU.

**p53 Knockout Mice (-/-) are More Susceptible than (+/-) or (+/+).**

Mutations of the p53 tumor suppressor gene constitute one of the most frequent molecular changes in a wide variety of human cancers. Mice deficient in p53 have recently attracted attention for their potential to identify chemical genotoxins. In this study we have investigated the susceptibility of p53 nullizygote (-/-), heterozygote (+/-) and wild-type (+/+) mice to MNU gastric carcinogenesis. p53 knockout mice were treated with 30 p.p.m. MNU in the drinking water 1 week on and 1 week off and killed after 5 weeks. The numbers of pepsinogen-altered pyloric glands (PAPG), putative preneoplastic lesions, were 1.8, 1.7 and 22.6 in p53 (+/+) and (-/-) animals, respectively. In a 15-week experiment, adenomas were found in 0 of 19 (+/+) (0%), 2 of 21 (+/-) (9.5%) and 6 of 10 (-/-) (60.0%) animals. Also, one well-differentiated adenocarcinoma was observed in a p53 (-/-) mouse. After 40 weeks treatment with 120 or 30 p.p.m. MNU there was no significant difference in the incidence of gastric tumors between p53 (+/+) and (-/-) mice. However, mortality from carcinogen-induced lymphomas, leukemias and sarcomas was very much greater in the latter group. Homozygous knockout animals could not be maintained long term. PCR-single strand conformation polymorphism analysis of exons 5–8 of the p53 gene of DNA extracts from 68 gastric tumors consistent with MNNG carcinogenesis induced by this direct acting agent.
References


