Review Article

Gastric Cancer: The Experimental Animal Model

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Gastric cancer (also called stomach cancer) can develop in any part of the stomach. With poor prognosis, gastric cancer causes nearly one million deaths worldwide per year. Many risk factors are involved in the development of gastric cancer, but the major one is *H. pylori* infection. In 1994, the World Health Organization and the International Agency for Research on Cancer classified *H. pylori* as a class I carcinogen. Histologically, gastric cancer was divided into two subtypes: intestinal-type and diffuse-type gastric carcinoma[1]. Intestinal-type gastric carcinoma is more common than the other. In addition, this subtype shows the progression of gastric cancer caused by *H. pylori* infection: normal gastric mucosa, *H. pylori*-induced gastritis, intestinal metaplasia, dysplasia, and gastric carcinoma[2].

Over recent years, a number of animal models have been developed, and the findings provided insights into the pathogenesis of gastric adenocarcinoma. It is important that mechanisms of *H. pylori* infection underlying the development of gastric cancer could help future cancer prevention. Therefore, the scope of this report is to focus on the epidemiology of gastric cancer and animal models that were developed gastric adenocarcinoma.

Epidemiology of gastric cancer

The most recent rate of the world-wide incidence of cancer shows that gastric cancer is the fourth most common cancer and the second leading cause of cancer-related death worldwide after lung cancer, with over 900,000 new cases diagnosed every year[3-5]. In 2000, about 880,000 people were diagnosed with gastric cancer and approximately 650,000 died of the disease[6]. By geographic variation throughout the world, gastric cancer incidence rates vary. Nearly two-thirds of stomach cancers occur in developing countries[6]. China, Japan, and Korea have the highest gastric cancer rates in the world, whereas Canada, USA, and UK have much lower rates. In addition, gastric cancer has been found in men more than in women. However, the factors leading to this variability among countries, races, and sex may be the genetic polymorphisms[7].

In Thailand, gastric cancer is one of the most common cancers and makes for 1-2 percent of all incident cancer[8]. Moreover, from the retrospectively reviewed, 119 patients with gastric cancer were recorded in King Chulalongkorn Memorial Hospital between January 1994 and December 1998[9]. Recently, it is reported that annual incidence rate of gastric cancer in Thailand is 7.1 per 100,000 populations[10].

Symptoms and diagnosis of gastric cancer

Gastric cancer is often asymptomatic or causes only nonspecific symptoms in its early stages. By the time symptoms occur, the cancer has generally metastasized to other parts of the body. Therefore, gastric cancer remains a disease of poor prognosis and high mortality. However, stomach cancer can cause the following signs and symptoms. Dyspepsia or loss of appetite (especially for meat) may be occurred at early. However, these symptoms may not sufficient for gastric cancer classification. At late stage of disease, the most common symptoms observed were abdominal mass, ascites, upper GI bleeding, and dramatically weight loss. To find the cause of symptoms, stomach

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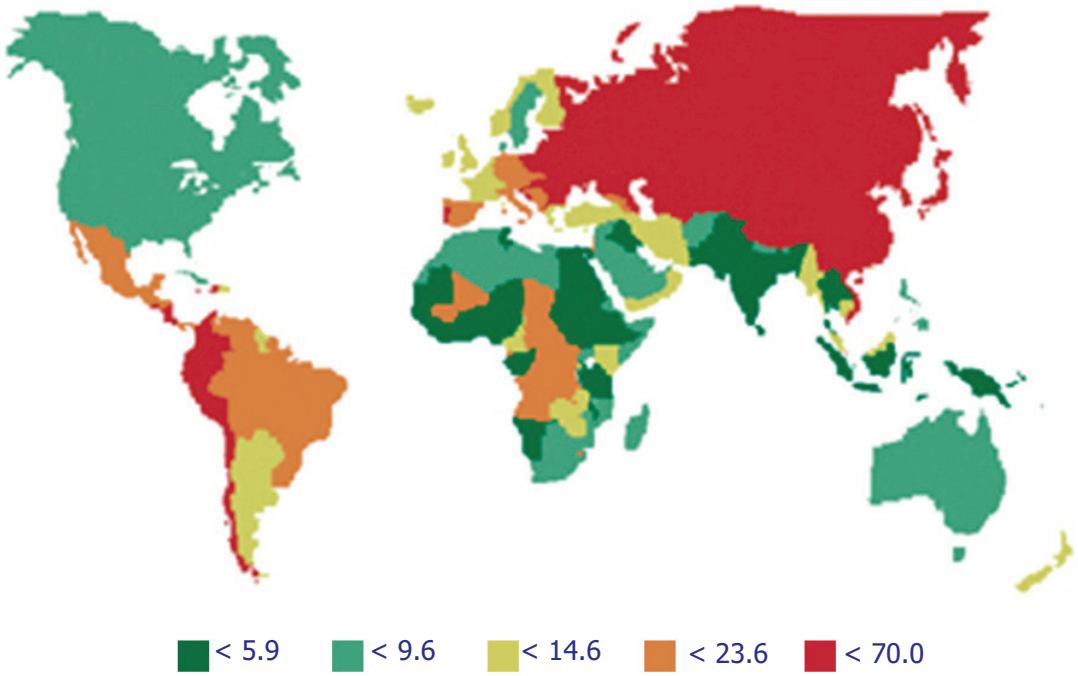

Figure 1. Epidemiology of gastric cancer (From Crew KD, *et al*. World J Gastroenterol 2006;12(3):354-62.)

tissue seen in gastroscope examination will be biopsied. Then, histological examination was used to determine the presence of cancer cells[9,11].

Experimental models for the study of gastric cancer

Gastric cancer is an important public health problem. To identify pathological and molecular biochemical mechanisms, various experimental models have been established. Rodent models are useful model of gastric adenocarcinoma. Before the discovery of *H. pylori* in 1982[12], rats were studied gastric cancer development by carcinogen induction such as benzo[a]pyrene, 3-methylcholanthrene, and 2-acethylaminofluorene[13-15]. However, the stomach cancer incidences of these experiments were low. In 1967, Sugimura and Fujimura[16] reported good yields of adenocarcinoma in the glandular stomachs of rats treated with N-methyl-N'-nitro-N-nitrosoguanidine (MNNG). After that MNNG was used for gastric cancer induction in many animal models such as dogs[17]. In mouse model of gastric cancer, Tatematsu *et al*. reported induction of good yields of adenocarcinoma in the glandular stomach of BALB/c[18] and C3H mice treated with N-methyl-N-nitrosourea (MNU). Because of the power of murine transgenic and knockout techniques, the mouse model has become the interested model to explain the pathophysiology of gastric cancer[19].

H. pylori is one of the most important factors for human stomach disorders, including gastric cancer. Many animals such as piglet[20], Beagle dog[21], and Japanese monkey[22] are successful *H. pylori* infection and used to study the pathologic background of *H. pylori* induced gastrointestinal diseases. However, none of these models infected with only *H. pylori* can develop gastric cancer.

The first and successful animal model developed intestinal metaplasia and gastric cancer resemble lesions apparent in human was the Mongolian gerbils (MG), in which infected with *H. pylori* alone[23-25]. The gastric cancer incidence was approximately 40% depending on study. In addition, the other factors were modified in the animal models. For example, a high-salt diet enhances the effects of *H. pylori* infection on stomach carcinogenesis, and these 2 factors act synergistically to promote the development of stomach cancers in the MG model[26]. In 1996, Furihata C, *et al*.[27] showed that gastric cancer incidence increase in a dose-dependent fashion of NaCl administration. It is possible that high salt diet upregulates the amount of surface mucous cell mucin, suitable for *H. pylori* colonization. Furthermore, *H. pylori* infection was found to increase the incidence of both MNU- and MNNG-in-

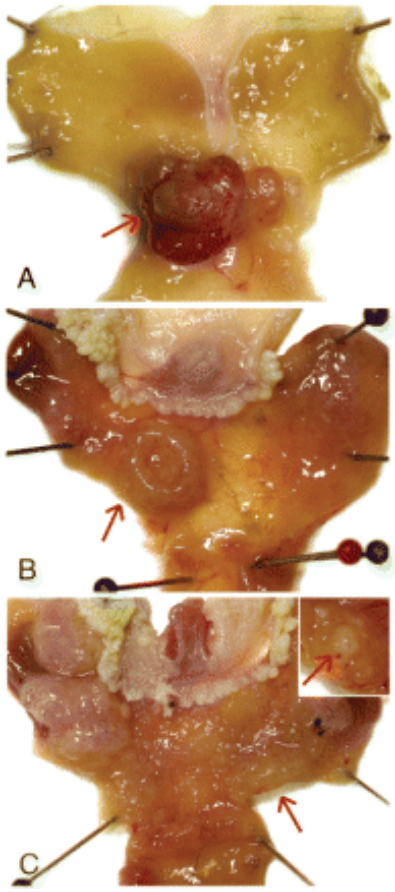

Figure 2. Macroscopic views of glandular stomach tumors. (A) A protruding type developing in an MNU-treated mouse. (B) Crater-shaped tumor in an MNU treated and *H. pylori* infected Mongolian gerbil. (C) Scirrhous type in an MNU treated and *H. pylori*-infected gerbil, difficult to identify in the mucosa but showing invasion to the serosa (inset). Tumors are indicated with red arrows. (From Tsukamoto T, *et al*. Toxicol Pathol 2007; 35(5):636-48.)

duced gastric adenocarcinoma in the MG (28-30). The MG infected with *H. pylori* and MNU- or MNNG-induced gastric cancer has proved very useful for the analysis of stomach carcinogenesis. Therefore, the study of gastric cancer development is mostly used MG treated with the carcinogen in *H. pylori*-infected stomach.

From the following studies, *H. pylori* infection promoted the MG become more susceptible to develop gastric adenocarcinoma. Shimizu *et al*.[31] reported that the incidence of gastric adenocarcinoma in MNU-administered MG with *H. pylori* infection (65%) was significantly higher than in MNU-administered MG that have *H. pylori* eradication (20%). Their results suggest that *H. pylori* eradication may prevent gastric carcinogenesis. In 2002, Cao *et al*.[32] designed an experiment to evaluate variation in susceptibility to stomach carcinogenesis in relation to age of acquisition of *H. pylori* infection. They used MG at the age of 4, 18, and 32 weeks for *H. pylori* inoculation followed by MNU treatment. 52 weeks after *H. pylori* inoculation, gastric cancer incidences in MG infected with *H. pylori* at the age of 4, 18, and 32 weeks were 60%, 18.4%, and 10%, respectively. Therefore, early infection of *H. pylori* significantly increases stomach cancer incidence, as compared to the case with later infection. It would be similar to human that childhood *H. pylori* infection enhances stomach cancer in adult life[33].

Recently, Cao *et al*.[34] hypothesized that early inoculation and long-term colonization of *H. pylori* would result in more severe chronic active gastritis, and therefore a greater yield of gastric carcinomas. They used seven-week-old male MG infected with *H. pylori* at experimental weeks 0, 12, or 18. At week 70, stom-

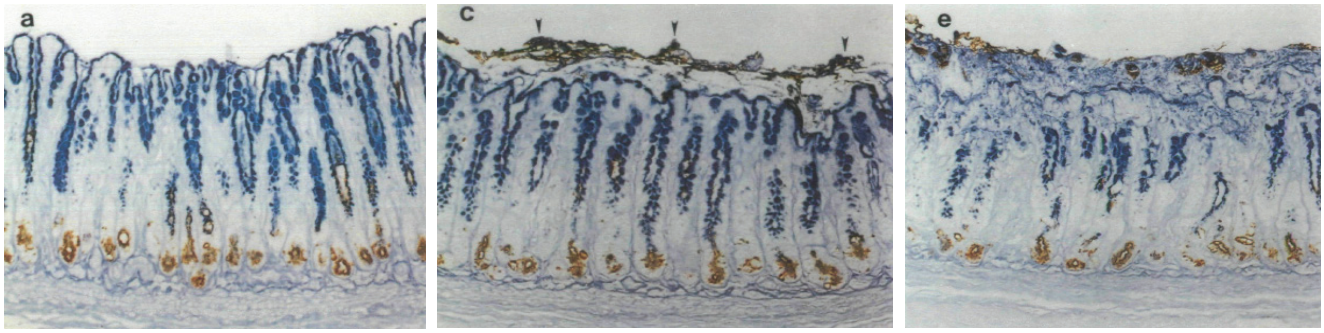

Figure 3. Administration of 1.3 M NaCl (2 ml) (c), and 3.7 M NaCl (0.7 ml) (e) induced concentration-dependent damage of the surface mucous cell layer after 1 min and increased replicative DNA synthesis after 17 h compare with control (a). (From Furihata C, *et al*. Carcinogenesis 1996;17(3):401-6.)

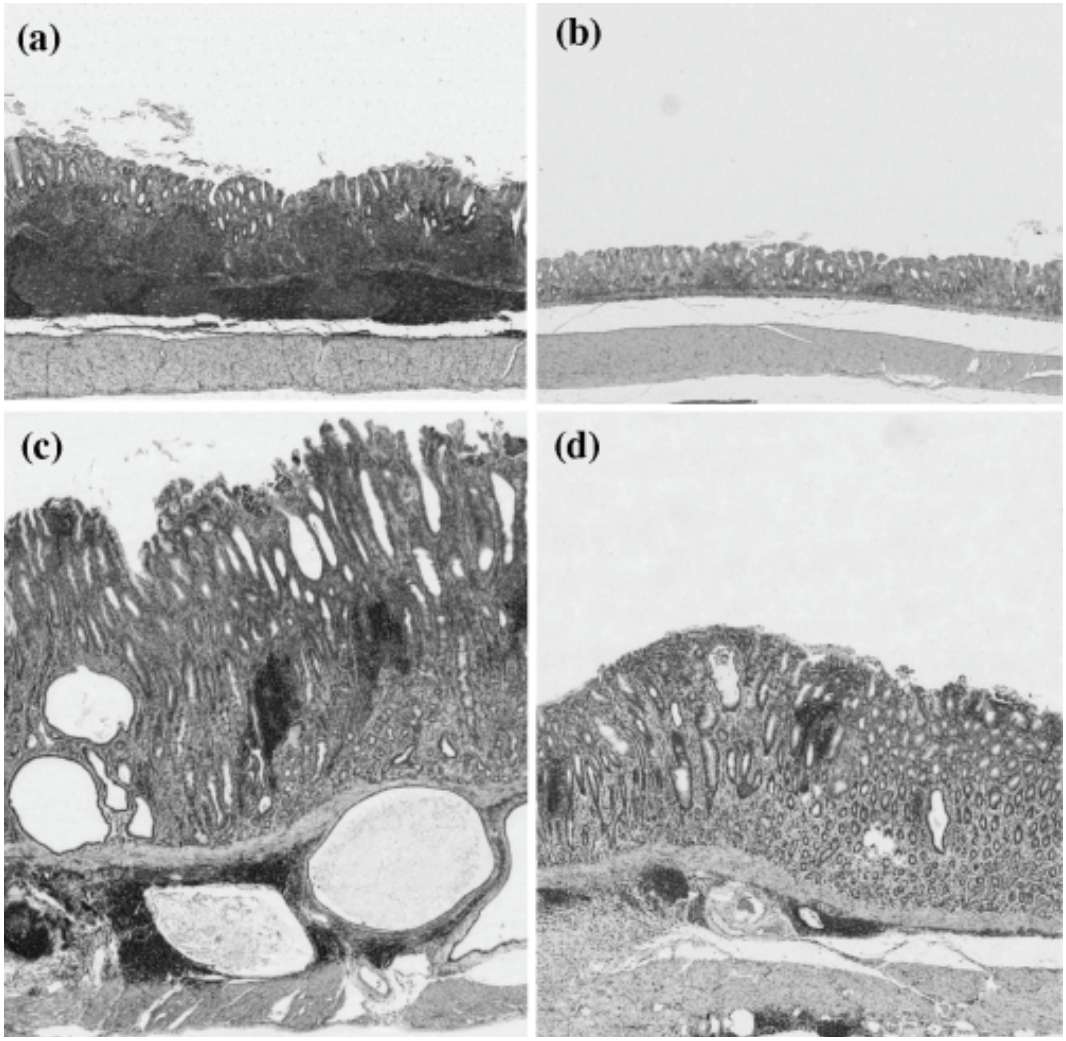

Figure 4. Histopathological findings of pyloric mucosa after inoculation of *H. pylori*. (a) Marked lymphoid follicle formation in a group a1 gerbil (H & E; × 25). (b) Mild gastritis in a group c1 gerbil (H & E; × 25). (c) Marked gastritis with heterotopic proliferative glands (HPGs) at 70 weeks post-infection (group A, H & E; × 25). (D) Moderate gastritis in a group C gerbil (H & E; × 25). (From Cao X, *et al*. Cancer Sci 2007;98(4):478-83.)

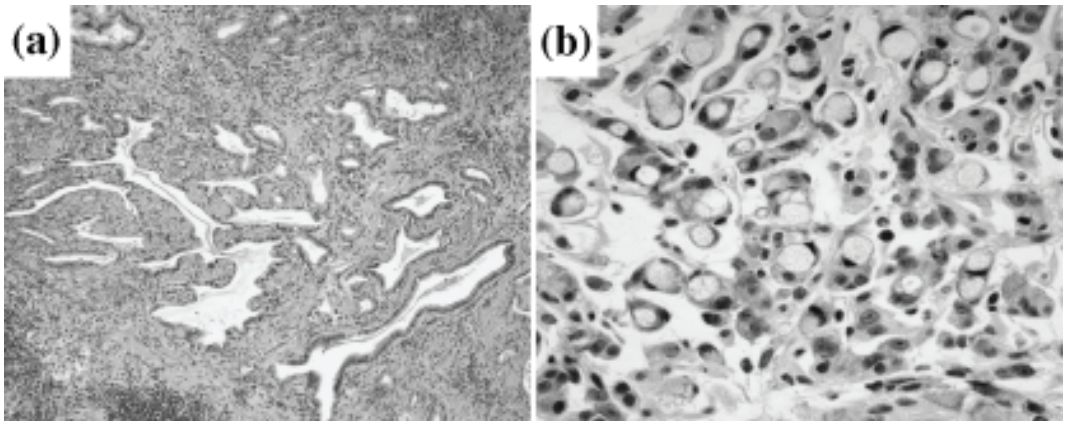

Figure 5. Histology of adenocarcinomas. (a) Well differentiated adenocarcinoma in glandular stomach at 70 weeks post-*H. pylori* infection in a group A gerbil (H & E; × 80). (b) Signet ring-cell carcinoma at 70 weeks post-infection in a group A gerbil (H & E; × 400). (From Cao X, *et al*. Cancer Sci 2007;98(4):478-83.)

achs were then excised for histological examination 70, 58, or 52 weeks after *H. pylori* inoculation, respectively. The respective incidences of glandular stomach adenocarcinoma were 65%, 20%, and 23%. The levels of interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and cyclooxygenase-2 (COX-2) mRNA were also examined by RT-PCR technique. The results showed that long-term *H. pylori* infection increased the expression of inflammatory mediators mRNA in the stomachs. Therefore, long-term *H. pylori* infection induced severity of gastric inflammation, and chronic inflammation provide the stomach become more sensitive for gastric carcinogenesis.

CONCLUSION

In this report, we presented the animal models that used for studying the pathology and mechanism underlying *H. pylori*-induced gastric cancer. From many successful *H. pylori*-infected animals, only Mongolian gerbil can develop gastric cancer. Moreover, *H. pylori* induced pathological changes in Mongolian gerbil is similar to that in human. Therefore, many studies use Mongolian gerbil as gastric cancer model. Furthermore, the carcinogen (MNNG or MNU) is used to increase the gastric cancer incidence in *H. pylori*-infected MG model. However, the other models, such as transgenic mice are also interested to study the possible mechanisms underlying *H. pylori*-associated gastric cancer. Thus, rodent models may be suitable for studying the pathology and biological mechanisms underlying gastric cancer development.

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