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• *REVIEW* •

Helicobacter pylori-infected animal models are extremely suitable for the investigation of gastric carcinogenesis

Masaaki Kodama, Kazunari Murakami, Ryugo Sato, Tadayoshi Okimoto, Akira Nishizono, Toshio Fujioka

Masaaki Kodama, Kazunari Murakami, Ryugo Sato, Tadayoshi Okimoto, Toshio Fujioka, Department of Gastroenterology, Faculty of Medicine, Oita University, Hasama-machi, Oita 879-5593, Japan
Akira Nishizono, Department of Infectious Diseases (Microbiology), Faculty of Medicine, Oita University, Hasama-machi, Oita 879-5593, Japan
Correspondence to: Masaaki Kodama, Department of Gastroenterology, Oita University, Faculty of Medicine, 1-1, Idaigaoka, Hasama-machi, Oita-gun, Oita 879-55, Japan. kodm@med.oita-u.ac.jp
Telephone: +81-975-86-6193 Fax: +81-975-86-6194
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Abstract

Although various animal models have been developed to clarify gastric carcinogenesis, apparent mechanism of gastric cancer was not clarified in recent years. Since the recognition of the pathogenicity of *Helicobacter pylori* (*H pylori*), several animal models with *H pylori* infection have been developed to confirm the association between *H pylori* and gastric cancer. Nonhuman primate and rodent models were suitable for this study. Japanese monkey model revealed atrophic gastritis and p53 mutation after long-term infection of *H pylori*. Mongolian gerbil model showed the development of gastric carcinoma with *H pylori* infection alone, as well as with combination of chemical carcinogens, such as *N*-methyl-*N*-nitrosourea and *N*-methyl-*N*-nitro-*N*'-nitrosoguanidine. The histopathological changes of these animal models after *H pylori* inoculation are closely similar to those in human beings with *H pylori* infection. Eradication therapy attenuated the development of gastric cancer in *H pylori*-infected Mongolian gerbil. Although several features of animal models differ from those seen in human beings, these experimental models provide a starting point for further studies to clarify the mechanism of gastric carcinogenesis as a result of *H pylori* infection and assist the planning of eradication therapy to prevent gastric carcinoma.

Key words: *Helicobacter pylori*; Gastric carcinoma; Animal model; Japanese monkey; Mongolian gerbil

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INTRODUCTION

Gastric cancer is one of the main causes of cancer-related mortality, especially, in East Asia. To clarify the mechanism of gastric cancer development, many experimental models have been used. However, almost all experimental animals, that showed spontaneous gastric cancer were very rare[1]; therefore several animal models were established using chemical carcinogens, such as *N*-methyl-*N*-nitrosourea (MNU)[2,3] and *N*-methyl-*N*-nitro-*N'*-nitrosoguanidine (MNNG)[4,5], which showed a high rate of gastric cancer development, especially in the antrum.

Since Warren and Marshall[6] revealed the microorganism which inhabits the stomach, *Helicobacter pylori* (*H pylori*) was considered as the major factor of many kind of gastroduodenal diseases, such as acute gastritis[7-9], chronic atrophic gastritis[9,10], intestinal metaplasia[11], peptic ulcer[12,13], mucosal associated lymphoid tissue lymphoma[14], gastric cancer[15-18], and others[19,20].

Previously, a large number of epidemiological studies indicated that *H pylori* infection has a close relation with gastric cancer[15-18]. Therefore, the International Agency for Research on Cancer (IARC) conference of the World Health Organization (WHO) defined *H pylori* as a definite carcinogen (Group I) to the human stomach based on three prospective case-control studies[15-17] reported in 1991[21].

However, the mechanisms by which *H pylori* infection develop gastric cancer are not defined in detail. In further studies, attempts have been made to reveal the possible mechanisms by which *H pylori* contributes to the development of gastric carcinoma and many researchers have developed animal models of infection using *Helicobacter* species.

Previously, a large number of animal experimental models have been developed to define the association between *H pylori* infection and gastroduodenal disease, such as piglet[22], beagle dog[23], mice[24], rhesus monkey[25], Japanese monkey (*Macaca fuscata*)[9,26,27], Mongolian gerbil[28], and others. In the beginning of the development of experimental models, only a few models had long periods of infection.

We have reported the results of a 5-year study on *H pylori* infection using Japanese monkeys (*Macaca fuscata*)[27]

and have obtained the findings that advance gastric mucosal atrophy, increase proliferation and mutation of p53 in gastric epithelial cells[29,30].

Several experiments, which demonstrated that chronic *H pylori* infection models of Mongolian gerbils developed gastric carcinoma, were conducted[31-33]. In these experiments, the animals were mainly divided into two groups: one group was infected with *H pylori* alone and the group was given a known carcinogen such as MNU and MNNG in addition to persistent *H pylori* infection. The results of these experiments revealed that animals in different groups developed different histopathological types of gastric carcinoma. These results will be very useful to elucidate the mechanism of gastric carcinogenesis due to *H pylori* infection.

GASTRIC CANCER AND JAPANESE MONKEY

The nonhuman primate animals are useful to clarify the relationship between *H pylori* and gastric diseases. Their stomachs are similar to those of human beings anatomically, physiologically, and dietary, compared with rodent animals. They have 10-20 years of long life span, which enables long-term follow-up with endoscopy and repeated histological examinations of the stomach using biopsy or endoscopic resected specimens. Several primate animals have been reported to be successful in experimental transmission of *H pylori* in chimpanzees (*Pan troglodytes*)[34], and species of macaques: rhesus monkey (*M. mulatta*)[25], cynomolgus monkey (*M. fascicularis*)[25], and Japanese monkey (*M. fuscata*)[9,26,27]. In these animals, some kinds of *Macaque* species are available for a wide variety of research field. We have established the Japanese monkey model with *H pylori* infection. This experimental model is very useful and a promising nonhuman primate model[9,26,27].

The methods of development of this monkey model are described briefly. The bacterial strains used were *H pylori* MCO 88155, MCO 88099, MCO 88142, and MCO 88156, isolated from two patients with duodenal ulcers and two with gastric ulcers. The colonies were suspended in 5 mL of sterile saline, and the bacterial concentration was adjusted to 10^9 CFU/mL. These were resuspended in 8 mL of sterile saline, and 5 mL of the final resuspension was used in each monkey. The animals were given ampicillin orally to eradicate spiral bacteria other than *H pylori*. After treatment with ampicillin, spiral bacteria were not found in any of the stomachs. The monkeys were sprayed with 5 mL of a mixed suspension of four bacterial strains endoscopically around their antrum. The gastric mucosa was examined endoscopically, and endoscopic mucosal resection was performed repeatedly during 6 years of observation.

One week after inoculation, all infected monkeys showed endoscopic acute gastritis accompanied by marked erythema and edema. These findings were consistent with the acute gastric mucosal lesion observed in the human stomach. Infection of *H pylori* was recognized by culture, the rapid urease test, histology, and the elevation of *H pylori*-specific IgG in plasma. In the early phase of infection, infiltration of monocytes and polymorphonuclear leukocytes were marked in the edematous lamina propria and superficial erosions were evident. After 3 mo of inoculation, infiltration of mononuclear cells and plasma cells were predominant in the lamina propria layer. However, no superficial erosions and atrophic changes were observed.

In the infected group, the gastritis score which was evaluated by a scoring system based on the method of Rauws *et al*[35]. were markedly increased in the antral mucosa 1 wk after inoculation ($P<0.001$). The score then gradually decreased throughout the whole investigation period, but remained significantly higher ($P<0.01$) than that of the control group.

Six months after inoculation, the pyloric glandular height was apparently lower in the infected animals than in controls. Furthermore, the atrophic change advanced gradually throughout the 5-year observation period[27]. Endoscopically, according to the endoscopic-atrophic-border scale described by Kimura and Takemoto[36], gastric atrophy also gradually advanced for more than 3 years. These findings indicated evidently that *H pylori* infection caused atrophic gastritis in the Japanese monkey model. Cell proliferation activity, which was revealed with immunohistochemical detection of Ki-67 in the antral mucosa of infected animals, was significantly accelerated throughout the entire observation period (Figure 1). Immunohistochemical detection of p53 and point mutation of *p53* was exhibited in the gastric mucosa[29,30] of this model. Genetic alterations in exons 5-8 of the p53 gene were uncommon in the *H pylori*-uninfected monkeys, whereas a higher prevalence of missense mutations in the p53 gene appeared in association with *H pylori* infection (Table 1). The number of mutations in the p53 gene increased as the gastric atrophy score increased, which depends on the duration of *H pylori* infection[30]. These findings of Japanese monkey model may explain the potential mechanism for the causal role of *H pylori* in the chain of events leading to gastric carcinoma. This monkey model facilitates investigation of the correlation between the long-term sequence of *H pylori* infection and gradual gastric mucosal change. Although many pathophysiological changes were seen in *H pylori*-infected gastric mucosa, this Japanese monkey model did not show the development of gastric carcinoma. In their long life span, which is similar to human beings, further continuous infection may be needed to the more dramatic histological change.

DEVELOPMENT OF THE RODENT MODEL

Several rodent models were established for examining the etiological feature of *Helicobacter* species infection, such as mice[24,37], rat[38], and Mongolian gerbil[28]. Compared with nonhuman primate models, rodent models are treated easily, and are economical.

Marchetti *et al*[39]. reported the several clinical isolates colonized the stomach of SPF conditioned mice (CD1 mice) and Balb/c mice; however, colonization was very

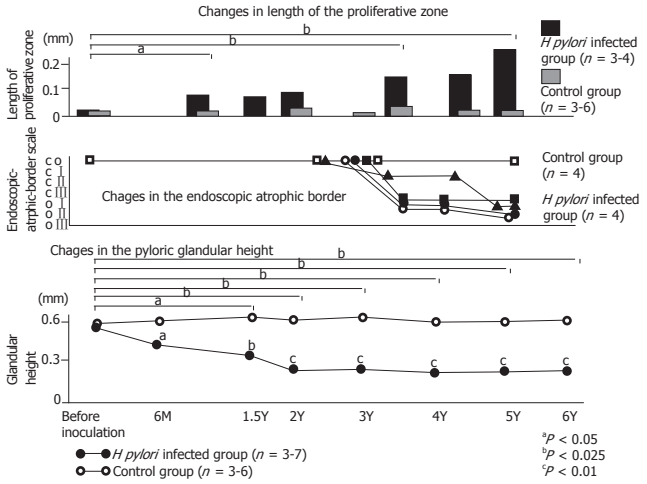

Figure 1 Gastric mucosal alteration of Japanese monkey model with *H pylori* infection. Upper graph showed the gradual increase of the proliferative zone of *H pylori*-infected Japanese monkey model. Middle graph showed the alteration of endoscopic-atrophic-border scale of this model. Macroscopically, gastric atrophy advanced for more than 3 yr. Lower graph showed the alteration of the pyloric glandular height. Six months after inoculation, the pyloric glandular height was apparently lower in the infected animals than in controls. Furthermore, the atrophic change advanced gradually throughout the 6-yr observation period.

Table 1 Duration of *H pylori* infection and number of point mutations in exon 5-8 of the p53 gene

Monkey	Duration of *H pylori* infection (yr)	Number of nucleotide (amino acid) substitutions in p53				Atrophy score[1]	Intensity of p53 immunostaining[2]
		Ex 5	Ex 6	Ex 7	Ex 8		
A	1.5	0 (0)	2 (1)	0 (0)	1 (1)	2	-
B	2	4 (0)	2 (2)	5 (4)	4 (3)	3	-
C	3	4 (2)	1 (0)	2 (2)	2 (2)	4	+
D	3	2 (1)	1 (0)	1 (1)	4 (2)	5	+
E	3.5	5 (2)	2 (1)	5 (4)	6 (4)	4	+
F	4.5	5 (1)	3 (1)	4 (3)	5 (4)	6	-
G	5	4 (1)	3 (1)	2 (1)	8 (6)	8	+
H	7.5	8 (5)	2 (1)	5 (4)	8 (4)	12	++

[1], The atrophy score was calculated as the sum of the histological evaluations of five gastric specimens according to Updated Sydney System. [2], The intensity of p53 immunostaining was classified into four grades: -, no staining; +, mild staining; ++, moderate staining; +++, intense staining.

low. Lee *et al*[40]. reported the quite good colonization by using the Sydney strain of *H pylori* (strain SS1), which is cagA and vacA positive.

These rodent models showed meager development of spontaneous gastric cancer. Cui *et al*[41]. reported the development of spontaneous gastric carcinoma, classified as malignant enterochromaffin-like (ECL) carcinomas in female cotton rats (*Sigmodon hispidus*). Previously, chemical carcinogens such as MNNG and MNU have been often used in the rodent species for the investigation of experimental gastric cancer[2-5].

From the recognition of *Helicobacter* species' pathogenicity, Fox *et al*[42]. reported a possible carcinogenic role for *Helicobacter* species in the gastric mucosa after oral administration of MNNG in ferrets infected with *Helicobacter mustelae*. Nine out of the ten ferrets, which were given a dose of 50 mg/kg MNNG orally, developed adenocarcinoma. This was the first experimental study using carcinogens combined with infection with a *Helicobacter* species. Although spontaneous gastric adenocarcinomas have been reported[43] in aged ferrets with *H mustelae* even in the absence of carcinogen exposure, an important additional problem is that the bacterium used was not *H pylori* but *H mustelae*. Fox *et al*[44]. also described the development of gastric adenocarcinoma, which was led from severe gastritis in C57BL/6 mice with *Helicobacter felis* inoculation. In their report, p53+/- mice showed significant low prevalence of fundic lymphoplasmacytic infiltration and submucosal lymphoid follicle formation than those in C57BL/6 mice. They indicated two distinct roles of p53, one of them displayed the gastric cancer risk. However, deletion of one *p53* allele results in a down-regulated Th1 response to *Helicobacter* infection, which may indirectly protect against the development of gastric cancer associated with chronic inflammation.

The differences between *H felis* in mice and *H pylori* in human beings are the lack of induction of neutrophil and cag pathogenicity island, which are recognized as main pathogens of *H pylori*. Kim *et al*[45]. reported that C57BL/6 mice infected with *H pylori* (SS1 strain) showed no evidence of gastric adenoma, dysplasia, and carcinoma during 80 wk of infection. They explained this result by the balance that exists between cell proliferation and apoptosis.

Transgenic hypergastrinemic (INS-GAS) mouse model have also been useful for the investigation of gastric carcinogenesis. Fox *et al*[46]. reported that male INS-GAS mice infected with *H pylori* developed atrophy, intestinal metaplasia, and dysplasia and adenocarcinoma. This murine model with *H pylori cagE* mutant showed the deletion of development of cancer. In contrast, none of the female mice with *H pylori* infection developed adenocarcinoma. However, IL-1 levels showed no significant difference between males and females. Fox *et al*. concluded that the INS-GAS model is effective for investigating discrete host-microbial interactions that culminate in gastric cancer within the context of biologic conditions induced by *H pylori*.

DEVELOPMENT OF MONGOLIAN GERBIL MODEL

Yokota *et al*[28]. developed the experimental Mongolian gerbil model with *H pylori* infection, in which only a mild inflammatory infiltration in the gastric mucosa was seen during two months of their observation. Hirayama *et al*[47]. described that ulcers and intestinal metaplasia were produced 6 mo after inoculation with *H pylori* in Mongolian gerbils.

In our laboratory, 5-wk-old male Mongolian gerbils weighing 30-40 g (Seiwa Experimental Animals Co. Ltd., Fukuoka, Japan) were used[48]. *H pylori* ATCC-43504 possessing the cagA gene and expressing vacuolating cytotoxin was used. A 4-d culture on blood agar at 37 ℃ under microaerophilic conditions was harvested and incubated in brucella broth (DIFCO Laboratories, Detroit,

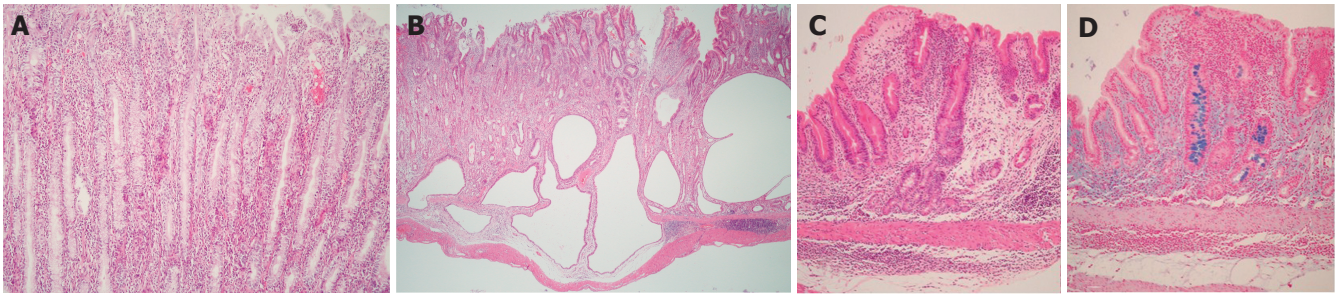

Figure 2 Microscopic views of the gastric body of Mongolian gerbils at 18 mo after *H pylori* inoculation. **A:** Severe infiltration of polymorphonuclear and mononuclear cells were seen in the lamina propria. (HE stain, x100); **B:** Some glands have extended into the submucosa but not into the proper muscularis layer. Severe infiltration of mononuclear cells in the submucosa (HE stain, x10); **C:** Intestinal metaplasia is seen scattering in gastric mucosa (HE stain, x10); **D:** Intestinal metaplasia (Alcian blue stain (pH 2.5); original magnification, x10).

MI, USA) with 10% horse serum for 24 h. Inoculum size was adjusted with sterile saline to produce the optical density of McFarland 4 at 540 nm. Mongolian gerbils were housed five per cage, starved for 24 h, and then fed with chow (Oriental Yeast Co., Tokyo, Japan) and water *ad libitum* beginning 12 h after *H pylori* inoculation. On the day of infection, the Mongolian gerbils were challenged orally with vehicle or 10^9 CFU *H pylori* in 1.0 mL of brucella broth with 10% horse serum. The spiral bacteria were observed in the mucus and gastric pits of all inoculated animals from 1 mo after inoculation throughout the whole observation period. However, nearly half of the animals had barely detectable *H pylori* in the stomach by bacterial culture. The bacterial counts from the stomachs of gerbils 1 and 6 mo after *H pylori* inoculation were 25 and 410 CFU/10 mg of gastric tissue, respectively[48]. These levels of colonized bacteria were nearly 1/10 to 1/100 than those of human being and monkey.

Mongolian gerbils with *H pylori* infection showed irregularly thickened gastric walls and spotty hemorrhages and erosions macroscopically, 1 year after inoculation. A severe infiltration of polymorphonuclear and mononuclear cells was seen in the lamina propria and mononuclear cells infiltration with lymphoid follicle in the submucosa, 1 mo after *H pylori* inoculation (Figure 2A). Erosion of the gastric mucosa appeared soon after inoculation, whereas gastric ulcers, gastritis cystica profunda (Figure 2B), and atrophy with goblet cell metaplasia (Figures 2C and D) occurred between 3 and 6 mo after inoculation[48,49]. Moreover, Suzuki *et al*[50]. reported that *H pylori* inoculation induced neutrophil followed by an increase in the level of lipid peroxidation and activated glutathione (antioxidant) turnover. These sequential changes of histological changes in gastric mucosa were quite similar to those observed in human beings. Therefore, Mongolian gerbil model may be useful to study the relationship between *H pylori* infection and gastric lesions, which include gastric malignancy.

GASTRIC CANCER AND MONGOLIAN GERBIL MODEL

Mongolian gerbils have also been induced by the development of gastric carcinoma with chemical carcinogen alone[51]. In addition, the results of several experimental studies have confirmed that administration of MNNG or MNU to Mongolian gerbils with chronic *H pylori* infection enhanced the development of different histopathological types of gastric carcinoma (Table 2)[31-33].

Sugiyama *et al*[31]. reported the development of carcinoma in the Mongolian gerbils evaluated at 40 wk after an experiment in which 7-wk-old animals were inoculated with *H pylori* (ATCC43504) and given 10 or 30 ppm MNU before or after inoculation. In this report, only the groups of the animals, which were administered with both *H pylori* and MNU, developed gastric cancers; more specifically, they developed different types of adenocarcinoma, such as well-differentiated, poorly differentiated, and signet ring cell carcinoma. These interesting experimental results support the results so far obtained in largescale epidemiological investigations[52].

The group inoculated with *H pylori* after being given MNU showed a distinctive initiation-promotion effect, whereas the group to which MNU was given after inoculation with *H pylori* appeared to demonstrate the simultaneous action of these two factors, with *H pylori* acting as a coinitiator. No gastric carcinoma was found within 40 wk of *H pylori* infection alone.

Tokieda *et al*[32]. conducted a study in which 5-wk-old Mongolian gerbils were inoculated with *H pylori* (ATCC43504) and orally given MNNG at 50 g/mL for 20 wk for comparison against animals administered with MNNG alone. At the 52nd wk after initiation, the group treated with MNNG and *H pylori* developed gastric carcinoma at a significantly higher frequency than the group treated with MNNG alone. In addition, cell proliferation was revealed to be markedly accelerated in those animals infected with *H pylori* with evaluation using a labeling index of 5-bromo-2'-deoxyuridine. This result suggests the possibility of explaining the link between *H pylori* infection and early events in gastric carcinogenesis. One of the interests in this study is that administration of MNNG reduced the infection rate of *H pylori* with the lapse of time, due to the likelihood of MNNG showing low-level (200 μg/mL) antibacterial activity against *H pylori*[32]. It is also of interest that *H pylori*-free animals did not develop gastric carcinoma even with

Table 2 Gastric carcinogenesis in *Helicobacter pylori*-infected Mongolian gerbils

Author	Year	Strain	Study design (ppm)	Incidence of cancer (%)	Duration of experiment (wk)
Sugiyama *et al*.	1998	ATCC43504	HP→MNU (10)	7/19 (36.8)	40
			HP alone	0/20 (0)	40
			MNU (30) → HP	6/18 (33.3)	40
			MNU alone	0/74 (0)	40
Tokieda *et al*.	1999	ATCC43504	HP → MNNG (50)	5/17 (29.4)	52
			persistent HP positive	5/8 (62.5)	52
			HP eradicated	0/9 (0)	52
			Br → MNNG (50)	3/22 (13.6)	50
Shimizu *et al*.	1999	ATCC43504	MNNG (300) → HP	12/27 (44.4)	50
			MNNG (300) → Br	1/19 (5.3)	50
			MNNG (60) → HP	6/25 (24.0)	50
			MNNG (60) → Br	0/20 (0)	50
			HP → MNNG (100)	4/27 (14.8)	50
			Br → MNNG (100)	3/18 (16.7)	50
			HP → MNNG (20)	15/25 (60)	50
			Br → MNNG (20)	1/20 (5)	50
			HP alone	0/20 (0)	50

HP, *Helicobacter pylori*; MNU, *N*-methyl-*N*-nitrosourea; MNNG, *N*-methyl-*N'*-nitroso-*N*-nitrosoguanidine; Br, Brucella broth.

Table 3 Gastric carcinogenesis in *Helicobacter pylori*-infected Mongolian gerbils

Author	Year	Strain	*cag*A gene	Vacuolating cytotoxin	Incidence of cancer (%)	Duration of experiment (wk)	Histological type of carcinoma
Watanabe *et al*.	1998	TN2GF4[1]	+	+	10/27 (37)	62	Well differentiated adenocarinoma
Honda *et al*.	1998	ATCC43504[2]	+	+	2/5 (40)	72	Well differentiated adenocarinoma
Hirayama *et al*.	1999	ATCC43504[2]	+	+	1/56 (1.8)	64	Poorly differentiated adenocarinoma
Ogura *et al*.	2000	TN2[2]	+	+	1/23 (4)	62	Well differentiated adenocarinoma
Zheng *et al*.	2004	ATCC43504[2]	+	+	3/17 (18)	84	Well differentiated adenocarinoma
		H pylori 161[3]	+	+			

[1]*H pylori* isolated from patient with gastric ulcer; [2]Type of strains; [3]*H pylori* isolated from patient with gastric adenocarcinoma.

MNNG administration. This result indicated a stronger carcinogenic role of *H pylori* infection. Although it has been reported by Sugiyama[31] that *H pylori* can persistently colonize the stomach of MNUtreated Mongolian gerbils, it is interesting that the two studies[32,33] report that MNNG administration eradicates *H pylori* infection, resulting in a reduction of its carcinogenic effects in the stomach. In *H pylori*-infected Mongolian gerbils with MNNG administration, duodenogastric reflux due to surgical procedure might attenuate the effect of *H pylori* on gastric tumorigenesis[53]. Because of our study, which indicated that bile reflux might lead to *H pylori* eradication[54], their results may probably depend on the *H pylori* eradication.

CARCINOGENICITY OF *H PYLORI* INFECTION ALONE

Although these studies showed marked increase of the chemical carcinogenic risk in the Mongolian gerbils, direct relationship between *H pylori* and gastric carcinogenesis was not indicated.

Two experimental studies attempted to confirm prior epidemiological studies that have demonstrated an association between *H pylori* infection and gastric carcinogenesis in human beings using Mongolian gerbils chronically infected with this bacterium (Table 3)[55,56]. Both studies confirmed gastric carcinogenesis resulting from *H pylori* infection alone, and were the first papers to fulfill Koch's postulates concerning *H pylori* infection and gastric carcinoma.

Watanabe *et al*[55]. used *H pylori* isolated from patients with gastric ulcer (TN2GF4), and Honda *et al*[56]. used ATCC43504 type strain, both of which were inoculated into 5-wk-old SPF Mongolian gerbils. The results showed that 37% (10 out of 27) of the animals in the former study developed well-differentiated adenocarcinoma at 62 wk after inoculation, whereas 40% (2 out of 5) of the animals in the latter study developed well-differentiated adenocarcinoma at 72 wk after inoculation (Figure 3). Both of these strains contained *cagA* and produced vacuolating cytotoxins. Sequential histopathological changes leading to carcinogenesis of the gastric mucosa were found to be common to the two studies, and very closely resembled the histopathological changes in human gastric mucosa caused by *H pylori* infection.

Hirayama *et al*[57]. reported that poorly differentiated adenocarcinoma and carcinoid were developed in

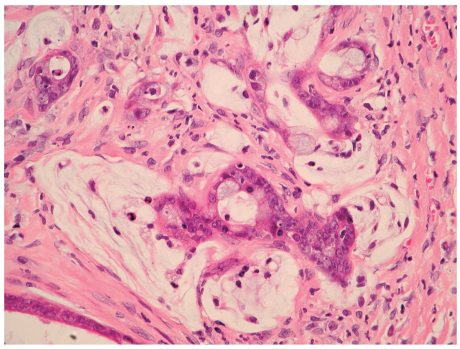

Figure 3 Microscopic views of the gastric mucosa of Mongolian gerbils at 18 mo after *H pylori* inoculation. Well-differentiated adenocarcinoma has extended into the muscular layer. Atypical glands and nuclei and abnormal mitosis are evident (HE stain, x40).

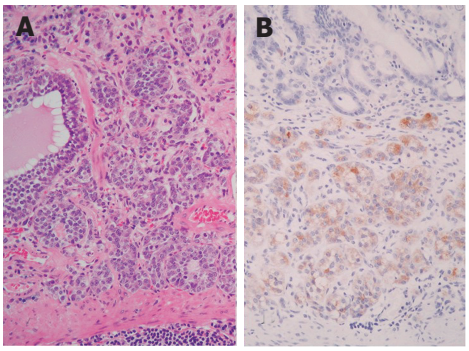

Figure 4 Gastric carcinoid in the stomach of a Mongolian gerbils colonized for 24 mo by *H pylori*. Microscopic view showing intramucosal carcinoid tumor (**A:** HE stain, x20; **B:** immunohistochemistry of chromogranin A, x20').

Mongolian gerbils model with *H pylori* (ATCC43504 type strain) infection alone. Zheng *et al*[58]. reported that Mongolian gerbils models, which were infected with *H pylori* (ATCC43504) and *H pylori* 161 (isolated from a Chinese patient with gastric adenocarcinoma) showed the development of well-differentiated adenocarcinoma (Table 3). Ogura *et al*[59]. reported the development of well-differentiated gastric cancer in wild type (TN2) and isogenic mutant of *vacA* (TN2Δ*vacA*) of Mongolian gerbil.

Mongolian gerbils' model also showed the development of gastric carcinoid[57,59,60]. In our laboratory, ECL cell tumors with marked atrophic gastritis and with hypergastrinemia were observed in the fundic gland area of infected Mongolian gerbils, 24 mo after inoculation (Figure 4); in contrast, adenocarcinoma developed in pyloric gland area. Histopathological findings of the entire observation period in Mongolian gerbils after *H pylori* inoculation are summarized in Table 4.

Ogura *et al*[59]. discussed the virulence factors of *H pylori* in Mongolian gerbils. Experimental gastric cancer derived in Mongolian gerbils with wild type of *H pylori* and *vacA* mutant infection, whereas *cagE* mutant induced far milder change of gastritis and induced no gastric cancer, which indicates the essential role of cagPAI in the gastric diseases with *H pylori* infection.

Table 4 Gastric carcinogenesis in *Helicobacter pylori*-infected Mongolian gerbils

Histropathological findings	Mo			
	6	12	18	24
Gastritis	5/5	4/4	5/5	10/10
Gastric ulcer	4/5	3/4	5/5	5/10
Atrophy	4/5	4/4	5/5	10/10
Intestinal metaplasia	2/5	3/4	5/5	10/10
Dysplasia	0/5	2/4	4/5	10/10
Gastric cancer	0/5	0/4	2/5	5/10
Gastric carcinoid	0/5	0/4	0/5	5/10

Data represent positive case/control Uninfected control. animals (n = 5 each) showed no abnormal findings.

Table 5 Relation between p53 and *H pylori* and gastric mucosal change (tentative opinion)

Condition	Human	Japanese monkey	Mongolian gerbil
H pylori and p53 overexpression (histology)	++	++	+[1]
H pylori and p53 point mutation	++	++	-
H pylori and atrophic gastritis	++	++	++
H pylori and intestinal metaplasia	++	-	++
H pylori and gastric cancer	+[2]	-	++

[1]The p53 overexpression was observed only in gastric cancer; [2]Not proven by interventional study; ++, strong evidence; +, weak evidence; -, no evidence

PREVENTION OF GASTRIC CARCINOMA BY ERADICATION OF *H PYLORI*

Shimizu *et al*[61]. reported that the incidence of adenocarcinomas in MNU-administered Mongolian gerbils with *H pylori* infection (15 out of 23) was significantly higher than in MNU-administered Mongolian gerbils that underwent *H pylori* eradication (5 out of 24). Their results suggest that *H pylori* eradication may prevent gastric carcinogenesis, and Mongolian gerbil's models have also been useful to study the prevention of gastric carcinogenesis in human beings.

DIFFERENCES BETWEEN ANIMAL MODELS AND HUMAN BEINGS

Although the Japanese monkey model and Mongolian gerbil model showed the similar change of human stomach that was infected with *H pylori*, several features of animal models differ from those seen in human beings. In Japanese monkey model intestinal metaplasia was not seen during the whole observation period. Severe gastritis and lymphoid follicular hyperplasia in the submucosal layer and gastritis cystica profunda, which are seen in Mongolian gerbils, are not observed in human gastric mucosa.

Table 5 shows our tentative opinion on p53 and *H pylori* infection in animal model and human beings. Although no gastric carcinoma developed in Japanese monkey model, Mongolian gerbil model showed gastric carcinoma resulting from *H pylori* infection alone. In human and Japanese monkey, both p53 immunostaining[29,62-64] and point mutations[30,65] were observed in *H pylori* infection. In

Mongolian gerbil, the p53 immunostaining was detected in gastric cancer but not in atrophic gastritis; moreover, there were no p53 mutations in exons 5 to 8 in infected gastric mucosa[66].

Suzuki *et al*[67]. reported that Mongolian gerbil model showed significant attenuation of apoptosis and promotion of cell proliferation than those seen in mice model with *H pylori* inoculation. Crabtree *et al*[68]. also described the differences of mucosal cytokine response between Mongolian gerbils and mice, and gender differences in the magnitude of cytokine response to *H pylori*. The differences of features between the species suggested that the pathogens of gastric diseases does not associate only with *H pylori* and may reflect in part other host factors.

Sonic hedgehog (Shh) is an important endometrial morphogenetic signal during the development of the vertebrate gut. Shh controls gastrointestinal patterning in general and gastric gland formation in particular. Suzuki *et al*[69]. reported that the long-term colonization of *H pylori* led to attenuation of Shh expression. Loss of Shh expression correlated with the loss of parietal cells, disturbed maturation of the mucous neck cell-zymogenic cell lineage. van den Brink *et al*[70]. described the loss of Shh expression in the intestinal metaplasia of the human stomach. Loss of Shh expression not only in intestinal metaplasia, but also in the tissue of *H pylori*-induced fundic gland atrophy is important for considering the possible link to preneoplastic lesion formation[69].

QUITE A NEW CONCEPT OF GASTRIC CANCER ORIGIN

In 2004, Houghton *et al*[71]. reported the innovative idea of gastric cancer origin with the usage of *H. felis*/C57BL/6 mouse model. Previously, tissue stem cells have been recognized as the origin of carcinoma. However, their study showed that bone marrow-derived cells (BMDCs) might also represent a potential source of malignancy. Female C57BL/6 mice after undergoing lethal irradiation were transplanted with bone marrow from male C57BL/*6JGtrosa26* (ROSA26), which was labeled with X-galactosidase or green fluorescent protein. In this model, gastric mucosal apoptosis increased at 6- 8 wk after *H. felis* inoculation. After 52 wk of inoculation, beta-galactosidase (gal) and trefoil factor2 (TFF2) positive cells increased gradually, then 90% of the gastric mucosa at the squamocolumnar junction was replaced with cells derived from the donor marrow. One year after infection, intramucosal carcinoma or high-grade gastrointestinal intraepithelial neoplasia were seen in these mice. No evidence of BMDC engraftment was seen in *H. felis* uninfected mice. Authors indicated that BMDC originated the epithelial cancer and the necessity of *Helicobacter* infection in this process.

CONCLUSION

In various experimental models, nonhuman primate and rodent models showed the variable evidences, which clarified the association between *H pylori* infection and gastric cancer.

Experiments developed using Mongolian gerbils have demonstrated that *H pylori* infection is clearly responsible for gastric carcinogenesis, and provide important confirmation of the statements issued by IARC/WHO. It will be of critical importance to extrapolate the sequential histopathological changes found in the Mongolian gerbil to lesions in the human gastric mucosa, since this model is proven to provide important pointers for the study of the mechanism of gastric carcinogenesis as a result of *H pylori* infection. While Koch's postulates for *H pylori* and gastric carcinoma have now been fulfilled, an important question to be addressed is why the Mongolian gerbil is the only species in which carcinogenesis has been experimentally induced by infection with *H pylori*.

REFERENCES

1 **Cui G**, Qvigstad G, Falkmer S, Sandvik AK, Kawase S, Waldum HL. Spontaneous ECLomas in cotton rats (Sigmodon hispidus): tumours occurring in hypoacidic/hypergastrinaemic animals with normal parietal cells. *Carcinogenesis* 2000; **21**: 23-27

2 **Fort L,** Taper HS, Brucher JM. Gastric carcinogenesis in rat induced by methylnitrosourea (MNU). Morphology, and histochemistry of nucleases. *Z Krebsforsch Klin Onkol Cancer Res Clin Oncol* 1974; **81:** 51-62

3 **Fujita M**, Taguchi T, Takami M, Usugane M, Takahashi A. Lung metastasis of canine gastric adenocarcinoma induced by N-methyl-N'-nitro-N-nitroguanidine. *Gan* 1975; **66**: 107-108

4 **Kartasheva LA**, Bykorez AI. Induction of stomach tumors in rats by N-methyl-N-nitroso-N1-nitroguanidine. *Vopr Onkol* 1975; **21:** 50-55

5 **Koestner AW**, Ruecker FA, Koestner A. Morphology and pathogenesis of tumors of the thymus and stomach in Sprague-Dawley rats following intragastric administration of methyl nitrosourea (MNU). *Int J Cancer* 1977; **20**: 418-426

6 **Marshall BJ**, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. *Lancet* 1984; **1:** 1311-1315

7 **Marshall BJ,** Armstrong JA, McGechie DB, Glancy RJ**.** Attempt to fulfil Koch's postulates for pyloric Campylobacter. *Med J Aust* 1985; **142**: 436-439

8 **Morris A**, Nicholson G. Ingestion of *Campylobacter pylori*dis causes gastritis and raised fasting gastric pH. *Am J Gastroenterol* 1987; **82**: 192-199

9 **Fujioka T**, Shuto R, Kodama R, Fujiyama K, Kubota T, Murakami K, Perparim K, Nasu M. Experimental model for chronic gastritis with *Helicobacter pylori:* long term follow-up study in *H pylori*-infected Japanese macaques. *Eur J Gastroenterol Hepatol* 1993; **5** (supple 1)**:** S73-S78

10 **Kuipers EJ**, Uyterlinde AM, Peña AS, Roosendaal R, Pals G, Nelis GF, Festen HP, Meuwissen SG. Long-term sequelae of *Helicobacter pylori* gastritis. *Lancet* 1995; **345**: 1525-1528

11 **Sakaki N**, Momma K, Egawa N, Yamada Y, Kan T, Ishiwata J. The influence of *Helicobacter pylori* infection on the progression of gastric mucosal atrophy and occurrence of gastric cancer. *Eur J Gastroenterol Hepatol* 1995; **7** Suppl 1**:** S59- S62

12 **Schubert TT**, Bologna SD, Nensey Y, Schubert AB, Mascha EJ, Ma CK. Ulcer risk factors: interactions between *Helicobacter pylori* infection, nonsteroidal use, and age. *Am J Med* 1993; **94**: 413-418

13 **Maaroos HI**, Kekki M, Vorobjova T, Salupere V, Sipponen P. Risk of recurrence of gastric ulcer, chronic gastritis, and grade of *Helicobacter pylori* colonization. A long-term follow-up study

of 25 patients. *Scand J Gastroenterol* 1994; **29**: 532-536
14 **Wotherspoon AC**, Doglioni C, Diss TC, Pan L, Moschini A, de Boni M, Isaacson PG. Regression of primary low-grade B-cell gastric lymphoma of mucosa-associated lymphoid tissue type after eradication of *Helicobacter pylori*. *Lancet* 1993; **342:** 575-577
15 **Forman D**, Newell DG, Fullerton F, Yarnell JW, Stacey AR, Wald N, Sitas F. Association between infection with *Helicobacter pylori* and risk of gastric cancer: evidence from a prospective investigation. *BMJ* 1991; **302**: 1302-1305
16 **Parsonnet J**, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, Sibley RK. *Helicobacter pylori* infection and the risk of gastric carcinoma. *N Engl J Med* 1991; **325**: 1127-1131
17 **Nomura A**, Stemmermann GN, Chyou PH, Kato I, Perez-Perez GI, Blaser MJ. *Helicobacter pylori* infection and gastric carcinoma among Japanese Americans in Hawaii. *N Engl J Med* 1991; **325**: 1132-1136
18 An international association between *Helicobacter pylori* infection and gastric cancer. The EUROGAST Study Group. *Lancet* 1993; **341:** 1359-1362
19 **Gasbarrini A**, Franceschi F, Tartaglione R, Landolfi R, Pola P, Gasbarrini G. Regression of autoimmune thrombocytopenia after eradication of *Helicobacter pylori*. *Lancet* 1998; **352**: 878
20 **Sato R**, Murakami K, Watanabe K, Okimoto T, Miyajima H, Ogata M, Ohtsuka E, Kodama M, Saburi Y, Fujioka T, Nasu M. Effect of *Helicobacter pylori* eradication on platelet recovery in patients with chronic idiopathic thrombocytopenic purpura. *Arch Intern Med* 2004; **164**: 1904-1907
21 **International Agency for Research on Cancer**: Infection with Schistosomes, Liver Flukes and *Helicobacter pylori* . IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. 1994; **61**: 218-220
22 **Krakowka S,** Morgan DR, Kraft WG, Leunk RD**.** Establishment of gastric Campylobacter pylori infection in the neonatal gnotobiotic piglet. *Infect Immun* 1987; **55**: 2789-2796
23 **Radin MJ**, Eaton KA, Krakowka S, Morgan DR, Lee A, Otto G, Fox J. *Helicobacter pylori* gastric infection in gnotobiotic beagle dogs. *Infect Immun* 1990; **58**: 2606-2612
24 **Karita M,** Kouchiyama T, Okita K, Nakazawa T**.** New small animal model for human gastric *Helicobacter pylori* infection: success in both nude and euthymic mice. *Am J Gastroenterol* 1991; **86**: 1596-1603
25 **Euler AR**, Zurenko GE, Moe JB, Ulrich RG, Yagi Y. Evaluation of two monkey species (*Macaca mulatta* and *Macaca fascicularis*) as possible models for human *Helicobacter pylori* disease. *J Clin Microbiol* 1990; **28**: 2285-2290
26 **Shuto R**, Fujioka T, Kubota T, Nasu M. Experimental gastritis induced by *Helicobacter pylori* in Japanese monkeys. *Infect Immun* 1993; **61:** 933-939
27 **Fujioka T**, Kodama R, Honda S, Guei-Hua G, Nishizono A, Nasu M. Long-term sequelae of experimental gastritis with *Helicobacter pylori*: a 5-year follow-up study. *J Clin Gastroenterol* 1997; **25 Suppl 1:** S8- S12
28 **Yokota K**, Kurebayashi Y, Takayama Y, Hayashi S, Isogai H, Isogai E, Imai K, Yabana T, Yachi A, Oguma K. Colonization of *Helicobacter pylori* in the gastric mucosa of Mongolian gerbils. *Microbiol Immunol* 1991; **35**: 475-480
29 **Kodama M**, Fujioka T, Kodama R, Takahashi K, Kubota T, Murakami K, Nasu M. p53 expression in gastric mucosa with *Helicobacter pylori* infection. *J Gastroenterol Hepatol* 1998; **13**: 215-219
30 **Oda T**, Murakami K, Nishizono A, Kodama M, Nasu M, Fujioka T. Long-term *Helicobacter pylori* infection in Japanese monkeys induces atrophic gastritis and accumulation of mutations in the p53 tumor suppressor gene. *Helicobacter* 2002; **7**: 143-151
31 **Sugiyama A**, Maruta F, Ikeno T, Ishida K, Kawasaki S, Katsuyama T, Shimizu N, Tatematsu M. *Helicobacter pylori* infection enhances *N*-methyl-*N*-nitrosourea-induced stomach carcinogenesis in the Mongolian gerbil. *Cancer Res* 1998; **58**: 2067-2069
32 **Tokieda M**, Honda S, Fujioka T, Nasu M. Effect of *Helicobacter pylori* infection on the *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine-induced gastric carcinogenesis in mongolian gerbils. *Carcinogenesis* 1999; **20**: 1261-1266
33 **Shimizu N**, Inada K, Nakanishi H, Tsukamoto T, Ikehara Y, Kaminishi M, Kuramoto S, Sugiyama A, Katsuyama T, Tatematsu M. *Helicobacter pylori* infection enhances glandular stomach carcinogenesis in Mongolian gerbils treated with chemical carcinogens. *Carcinogenesis* 1999; **20**: 669-676
34 **Hazell SL**, Eichberg JW, Lee DR, Alpert L, Evans DG, Evans DJ, Graham DY. Selection of the chimpanzee over the baboon as a model for *Helicobacter pylori* infection. *Gastroenterology* 1992; **103**: 848-854
35 **Rauws EA,** Langenberg W, Houthoff HJ, Zanen HC, Tytgat GN. Campylobacter pyloridis-associated chronic active antral gastritis. A prospective study of its prevalence and the effects of antibacterial and antiulcer treatment. *Gastroenterology* 1988; **94:** 33-40
36 **KimuraK**, Takemoto T. Endoscopic atrophy border. *Endoscopy* 1969; **1**: 1-3
37 **Lee A,** Fox JG, Otto G, Murphy J. A small animal model of human *Helicobacter pylori* active chronic gastritis. *Gastroenterology* 1990; **99**: 1315-1323
38 **Danon SJ**, Moss ND, Larsson H, Arvidsson S, Ottosson S, Dixon MF, Lee A. Gastrin release and gastric acid secretion in the rat infected with either *Helicobacter* felis or *Helicobacter heilmannii*. *J Gastroenterol Hepatol* 1998; **13**: 95-103
39 **Marchetti M**, Aricò B, Burroni D, Figura N, Rappuoli R, Ghiara P. Development of a mouse model of *Helicobacter pylori* infection that mimics human disease. *Science* 1995; **267**: 1655-1658
40 **Lee A,** O'Rourke J, De Ungria MC, Robertson B, Daskalopoulos G, Dixon MF. A standardized mouse model of *Helicobacter pylori* infection: introducing the Sydney strain. *Gastroenterology* 1997; **112**: 1386-1397
41 **Cui G**, Qvigstad G, Falkmer S, Sandvik AK, Kawase S, Waldum HL. Spontaneous ECLomas in cotton rats (Sigmodon hispidus): tumours occurring in hypoacidic/hypergastrinaemic animals with normal parietal cells. *Carcinogenesis* 2000; **21**: 23-27
42 **Fox JG**, Wishnok JS, Murphy JC, Tannenbaum SR, Correa P. MNNG-induced gastric carcinoma in ferrets infected with Helicobacter mustelae. *Carcinogenesis* 1993; **14:** 1957-1961
43 **Fox JG,** Dangler CA, Sager W, Borkowski R, Gliatto JM. Helicobacter mustelae-associated gastric adenocarcinoma in ferrets (Mustela putorius furo). *Vet Pathol* 1997; **34:** 225-229
44 **Fox JG,** Sheppard BJ, Dangler CA, Whary MT, Ihrig M, Wang TC. Germ-line p53-targeted disruption inhibits *helicobacter*-induced premalignant lesions and invasive gastric carcinoma through down-regulation of Th1 proinflammatory responses. *Cancer Res* 2002; **62**: 696-702
45 **Kim DH,** Kim SW, Song YJ, Oh TY, Han SU, Kim YB, Joo HJ, Cho YK, Kim DY, Cho SW, Kim MW, Kim JH, Hahm KB. Long-term evaluation of mice model infected with *Helicobacter pylori*: focus on gastric pathology including gastric cancer. *Aliment Pharmacol Ther* 2003; **18** Suppl 1: 14-23
46 **Fox JG,** Wang TC, Rogers AB, Poutahidis T, Ge Z, Taylor N, Dangler CA, Israel DA, Krishna U, Gaus K, Peek RM Jr. Host and microbial constituents influence *Helicobacter pylori*-induced cancer in a murine model of hypergastrinemia. *Gastroenterology* 2003; **124**: 1879-1890
47 **Hirayama F**, Takagi S, Kusuhara H, Iwao E, Yokoyama Y, Ikeda Y. Induction of gastric ulcer and intestinal metaplasia in mongolian gerbils infected with *Helicobacter pylori*. *J Gastroenterol* 1996; **31**: 755-757
48 **Honda S**, Fujioka T, Tokieda T, Gotoh T, Nishizono A, Nasu M. Gastric ulcer, atrophic gastritis, and intestinal metaplasia caused by *Helicobacter pylori* infection in Mongolian gerbils. *Scand J Gastroenterol* 1998; **33**: 454-60
49 **Ikeno T**, Ota H, Sugiyama A, Ishida K, Katsuyama T, Genta RM, Kawasaki S. *Helicobacter pylori*-induced chronic active

gastritis, intestinal metaplasia, and gastric ulcer in Mongolian gerbils. *Am J Pathol* 1999; **154:** 951-960

50 **Suzuki H**, Mori M, Seto K, Kai A, Kawaguchi C, Suzuki M, Suematsu M, Yoneta T, Miura S, Ishii H. *Helicobacter pylori*-associated gastric pro- and antioxidant formation in Mongolian gerbils. *Free Radic Biol Med* 1999; **26**: 679-684

51 **Tatematsu M**, Yamamoto M, Shimizu N, Yoshikawa A, Fukami H, Kaminishi M, Oohara T, Sugiyama A, Ikeno T. Induction of glandular stomach cancers in Helicobacter pylori-sensitive Mongolian gerbils treated with N-methyl-N-nitrosourea and N-methyl-N′-nitro-N-nitrosoguanidine in drinking water. *Jpn J Cancer Res* 1998; **89:** 97-104

52 **Huang JQ**, Sridhar S, Chen Y, Hunt RH. Meta-analysis of the relationship between *Helicobacter pylori* seropositivity and gastric cancer. *Gastroenterology* 1998; **114**: 1169-1179

53 **Tanaka Y**, Osugi H, Morimura K, Takemura M, Ueno M, Kaneko M, Fukushima S, Kinoshita H. Effect of duodenogastric reflux on N-methyl-N′-nitro-N-nitrosoguanidine-induced glandular stomach tumorigenesis in Helicobacter pylori-infected Mongolian gerbils. *Oncol Rep* 2004; **11:** 965-971

54 **Abe H**, Murakami K, Satoh S, Sato R, Kodama M, Arita T, Fujioka T. Influence of bile reflux and *Helicobacter pylori* infection on gastritis in the remnant gastric mucosa after distal gastrectomy. *J Gastroenterol* 2005; **40**: 563-569

55 **Watanabe T**, Tada M, Nagai H, Sasaki S, Nakao M. *Helicobacter pylori* infection induces gastric cancer in mongolian gerbils. *Gastroenterology* 1998; **115:** 642-648

56 **Honda S**, Fujioka T, Tokieda M, Satoh R, Nishizono A, Nasu M. Development of *Helicobacter pylori* induced gastric carcinoma in Mongolian gerbils. *Cancer Res* 1998; **58**: 4255-4259

57 **Hirayama F**, Takagi S, Iwao E, Yokoyama Y, Haga K, Hanada S. Development of poorly differentiated adenocarcinoma and carcinoid due to long-term *Helicobacter pylori* colonization in Mongolian gerbils. *J Gastroenterol* 1999; **34**: 450-454

58 **Zheng Q**, Chen XY, Shi Y, Xiao SD. Development of gastric adenocarcinoma in Mongolian gerbils after long-term infection with *Helicobacter pylori*. *J Gastroenterol Hepatol* 2004; **19**: 1192-1198

59 **Ogura K**, Maeda S, Nakao M, Watanabe T, Tada M, Kyutoku T, Yoshida H, Shiratori Y, Omata M. Virulence factors of *Helicobacter pylori* responsible for gastric diseases in Mongolian gerbil. *J Exp Med* 2000; **192:** 1601-1610

60 **Kagawa J**, Honda S, Kodama M, Sato R, Murakami K, Fujioka T. Enterocromaffin-like cell tumor induced by *Helicobacter pylori* infection in Mongolian gerbils. *Helicobacter* 2002; **7**: 390-397

61 **Shimizu N**, Ikehara Y, Inada K, Nakanishi H, Tsukamoto T, Nozaki K, Kaminishi M, Kuramoto S, Sugiyama A, Katsuyama T, Tatematsu M. Eradication diminishes enhancing effects of *Helicobacter pylori* infection on glandular stomach carcinogenesis in Mongolian gerbils. *Cancer Res* 2000; **60**: 1512-1514

62 **Hibi K**, Mitomi H, Koizumi W, Tanabe S, Saigenji K, Okayasu I. Enhanced cellular proliferation and p53 accumulation in gastric mucosa chronically infected with *Helicobacter pylori*. *Am J Clin Pathol* 1997; **108**: 26-34

63 **Satoh K**, Kihira K, Kawata H, Tokumaru K, Kumakura Y, Ishino Y, Kawakami S, Inoue K, Kojima T, Satoh Y, Mutoh H, Sugano K. p53 expression in the gastric mucosa before and after eradication of *Helicobacter pylori*. *Helicobacter* 2001; **6**: 31-36

64 **Kodama M**, Fujioka T, Murakami K, Okimoto T, Sato R, Watanabe K, Nasu M. Eradication of *Helicobacter pylori* reduced the immunohistochemical detection of p53 and MDM2 in gastric mucosa. *J Gastroenterol Hepatol* 2005; **20**: 941-946

65 **Murakami K**, Fujioka T, Okimoto T, Mitsuishi Y, Oda T, Nishizono A, Nasu M. Analysis of p53 gene mutations in *Helicobacter pylori*-associated gastritis mucosa in endoscopic biopsy specimens. *Scand J Gastroenterol* 1999; **34**: 474-477

66 **Murakami K**, Fujioka T, Kodama M, Honda S, Okimoto T, Oda T, Nishizono A, Sato R, Kubota T, Kagawa J, Nasu M. Analysis of p53 mutations and *Helicobacter pylori* infection in human and animal models. *J Gastroenterol* 2002; **37 Suppl 13:** 1-5

67 **Suzuki H**, Miyazawa M, Nagahashi S, Mori M, Seto K, Kai A, Suzuki M, Miura S, Ishii H. Attenuated apoptosis in *H pylori*-colonized gastric mucosa of Mongolian gerbils in comparison with mice. *Dig Dis Sci* 2002; **47:** 90-99

68 **Crabtree JE**, Court M, Aboshkiwa MA, Jeremy AH, Dixon MF, Robinson PA. Gastric mucosal cytokine and epithelial cell responses to *Helicobacter pylori* infection in Mongolian gerbils. *J Pathol* 2004; **202**: 197-207

69 **Suzuki H**, Minegishi Y, Nomoto Y, Ota T, Masaoka T, van den Brink GR, Hibi T. Down-regulation of a morphogen (sonic hedgehog) gradient in the gastric epithelium of *Helicobacter pylori*-infected Mongolian gerbils. *J Pathol* 2005; **206**: 186-197

70 **van den Brink GR**, Hardwick JC, Nielsen C, Xu C, ten Kate FJ, Glickman J, van Deventer SJ, Roberts DJ, Peppelenbosch MP. Sonic hedgehog expression correlates with fundic gland differentiation in the adult gastrointestinal tract. *Gut* 2002; **51**: 628-633

71 **Houghton J**, Stoicov C, Nomura S, Rogers AB, Carlson J, Li H, Cai X, Fox JG, Goldenring JR, Wang TC. Gastric cancer originating from bone marrow-derived cells. *Science* 2004; **306**: 1568-1571

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