Introduction

Identification of the pathogenic role of H. pylori infection has revolutionized the approach toward chronic gastritis, peptic ulcer disease, gastric MALT (mucosa associated lymphoid tissue) lymphoma and gastric cancer. These diseases often result from infection of H. pylori, the accompanying host inflammatory response and the interaction with environmental factors. Eradication treatment against peptic ulcer disease is now approved by governmental health care system in Japan. Concerning gastric cancer, persistent histologic gastritis, followed by glandular atrophy, makes up a high background for development of gastric cancer and it is incapable by itself of clearing the infection without effective combined antibiotic treatment.

Gastric cancer is one of the most common malignancies in the world, although the incidence and mortality rate has been decreasing in recent decades. Gastric carcinogenesis is multi-factorial, and some environmental factors are involved in this process, such as excessive intake of salt, N-nitroso compounds in foods, and low consumption of fresh fruits and vegetables. H. pylori have been shown to be associated with an increased risk of gastric cancer in a number of epidemiological studies. On the basis of those epidemiological findings, the International Agency for Research on Cancer, World Health Organization (IARC/WHO) concluded in 1994 that H. pylori infection had a causal link to gastric carcinogenesis and is a definite carcinogen in humans. Following the statements, evidences that H. pylori infection leads to the development of gastric cancer have been accumulated in vivo and in vitro study. In this review, current progresses and issues on the animal model using Mongolian gerbil (MG) and in vitro study of the epithelial cell response to H. pylori infection will be discussed.

Development of Gastric Cancer in Animal Model

IARC/WHO conclusion was based on the results of several sero-epidemiological studies in humans. Geographical studies and case-control studies have demonstrated only an association between the prevalence of H. pylori infection and gastric cancer, not a causal relation between them. To obtain direct evidences of a causal relationship between H. pylori infection and occurrence of gastric cancer, clinical intervention studies are required in which eradication of H. pylori leads to a reduction in the occurrence of gastric cancer in humans, or an experimental observation of gastric cancer in animal model infected with H. pylori is necessary. In the first decade following the discovery of this organism, inoculation of H. pylori was reported to induce gastritis in gnotobiotic piglets, beagle dogs and Japanese monkeys. These animal models indicated that H. pylori infection could induce histologic gastritis, characterized by infiltration of numerous inflammatory cells, epithelial erosion and degeneration, and the Japanese monkey model demonstrated that H. pylori infection can induce gastric atrophy at 1.5 years after inoculation. However, these animals are too large to conduct cancer experiments, and there are still no reports on the development of gastric cancer in these animal models. Although a mouse model that resembles human H. pylori chronic gastritis was developed, a specialized H. pylori strain, SS-1 strain, or H. felis were used instead of H. pylori in studies using this model. Hirayama et al. reported for the first time in 1996 that H. pylori could induce gastritis, gastric ulceration and intestinal metaplasia during long-term infection in MG model. In this model, H. pylori was able to colonize in the stomach and induce gastritis at 12 weeks after inoculation, gastric ulceration at 24 weeks after inoculation, intestinal metaplasia at 24 to 48 weeks after inoculation. These histologic characteristics, infiltration of numerous neutrophils and lymphocytes, deep defects of gastric mucosal tissues leading the muscular layer and occurrence of intestinal metaplasia, resembled those of H. pylori infection in humans. Following this report, the MG model began to be used in experiments to study gastric carcinogenesis caused by H. pylori infection. In 1998, three papers on the development of gastric cancer using MG models were published in Japan, and
Table 1 Development of Gastric Cancer in H. pylori-infected Mongolian Gerbils

<table>
<thead>
<tr>
<th>Reports (year)</th>
<th>H. pylori strain</th>
<th>Chemical carcinogen</th>
<th>Observation at</th>
<th>Incidence (%)</th>
<th>Histology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sugiyama (1998)</td>
<td>ATCC 43504</td>
<td>MNU</td>
<td>40 weeks</td>
<td>13/37 (35%)</td>
<td>6 signet</td>
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<td></td>
<td></td>
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<td>2 poorly</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>5 well</td>
</tr>
<tr>
<td>Watanabe (1998)</td>
<td>TN2GF4</td>
<td>none</td>
<td>62 weeks</td>
<td>10/27 (37%)</td>
<td>10 well</td>
</tr>
<tr>
<td>Honda (1998)</td>
<td>ATCC 43504</td>
<td>none</td>
<td>72 weeks</td>
<td>2/5 (40%)</td>
<td>2 well</td>
</tr>
<tr>
<td>Hirayama (1998)</td>
<td>ATCC 43504</td>
<td>none</td>
<td>96 weeks</td>
<td>1/56 (1.8%)</td>
<td>1 poorly</td>
</tr>
<tr>
<td>Shimizu (1999)</td>
<td>ATCC 43504</td>
<td>MNNG</td>
<td>50 weeks</td>
<td>15/25 (60%)</td>
<td>4 signet</td>
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<td></td>
<td>9 well</td>
</tr>
<tr>
<td>Tokieda (1999)</td>
<td>ATCC 43504</td>
<td>MNNG</td>
<td>52 weeks</td>
<td>4/6 (67%)</td>
<td>4 well-moderate</td>
</tr>
</tbody>
</table>

(signet; signet ring cell carcinoma, poorly; poorly differentiated adenocarcinoma, well; well differentiated adenocarcinoma)

these findings strongly suggested that H. pylori infection could be responsible for the development of gastric cancer in animal experiments. Sugiyama et al.\textsuperscript{15} first demonstrated that H. pylori could increase the incidence of MNU-induced gastric cancer in MG animal models. A total of 170 MGs were used in their study. Seven of 19 MGs (36.8%) that had been inoculated with H. pylori ATCC type strain and then treated with 10 ppm of MNU for 20 weeks developed gastric cancers at 40 weeks. Five of the 7 cancers (71.4%) were signet ring-cell carcinomas, one was poorly differentiated adenocarcinoma, and one was well differentiated adenocarcinoma. In 6 of 18 MGs (33.3%) that had first been treated with 30 ppm of MNU for 6 weeks and then inoculated with H. pylori developed gastric cancers at 40 weeks. Four of the 6 cancers (66.6%) were well differentiated adenocarcinomas, one was signet ring-cell carcinoma and was one poorly differentiated adenocarcinoma. These findings may suggest that the time of inoculation of H. pylori, the dose of chemical carcinogens, and the order of inoculation of H. pylori and administration of chemical carcinogens are critical factors determining the histological types of gastric cancers. However, speculations concerning these factors, have not been confirmed in the other animal experiments using MG models. In contrast, 20 MGs infected with H. pylori ATCC type strain alone, and 18 MGs treated with 10 ppm of MNU alone for 20 weeks or 18 MGs treated with 30 ppm of MNU alone for 6 weeks did not demonstrate gastric cancer at all. In this finding, H. pylori infection plus administration of very low-dose chemical carcinogens was needed to develop gastric cancer in MG models.

In the same year, Watanabe et al.\textsuperscript{16} reported that long-term infection with H. pylori alone could induce gastric cancers in MG model at 62 weeks after the inoculation, without administration of any chemical carcinogens. They reported that 10 of 27 H. pylori-infected MGs (37%) developed gastric cancer and that all of them were well-differentiated, intestinal type gastric cancers. Interestingly, they used a very unique strain of H. pylori, named TN2GF4, which was originally isolated from a patient with gastric ulcer, then inoculated into the stomach of MG several times, and isolated again and used in their experiments. This strain had vacuolating cytotoxin and cagA gene, and it showed an extremely spiral form. Another key point in their study is that no gastric cancers were observed in the infected animals at 39 weeks or at 52 weeks after inoculation of H. pylori. Honda et al.\textsuperscript{17} also reported in the same year that 2 of 5 MGs (40%) infected with H. pylori ATCC 43504 type strain, having vacuolating cytotoxin and cagA gene, developed gastric cancer at 72 weeks after inoculation and that all of the cancers were well-differentiated cancers. However, Hirayama et al.\textsuperscript{18} reported that only one gastric cancer had developed in MGs infected with H. pylori ATCC 43504 type strain at 96 weeks follow-up (1.8%) and that the pathology was poorly differentiated adenocarcinoma.

The above-described studies were conducted under different experimental conditions, including different co-treatments of chemical carcinogens, strain differences, and different observation periods. These differences may have been the reasons for the differences in the incidence of gastric cancer and subtypes of gastric cancer (Table 1). The most troublesome issue is diagnostic criteria for gastric cancer in MGs. In general, gastric cancer in humans is diagnosed on the basis of three histological characteristics: 1) cellular and nuclear atypia, 2) aberrant glandular structure, and 3) invasion. H. pylori-infected MGs sometimes show invasion of glands into the muscular layer and aberrant glands, and these histologic changes are reversed with H. pylori eradication.\textsuperscript{19} These histologic characteristics in this model confuse us with well-differentiated cancers that developed in experiments in which animals were in-
fectected with *H. pylori* alone. On the other hand, treatment with low-dose chemical carcinogens sometimes induces cellular and nuclear atypia. Therefore, prudence is needed for diagnosis of well-differentiated adenocarcinoma in MGs induced by *H. pylori* infection. To resolve these issues, common criteria for diagnosis of gastric cancer in MGs is required. An alternative scientific approach is to find genetic evidence to confirm the diagnosis on gastric cancer.

**Genetic Alterations in Gastric Cancer and Animal Model**

Gastric carcinogenesis in humans is a multi-step. An accumulation of genetic alterations may result in development of gastric cancer, having an analogy with that of colon cancer. Genetic alterations occur in oncogenes, tumor suppressor genes, cell adhesion molecule genes, telomere and telomerase activities and the related genes and genetic instability in human gastric cancers. Different histological types of gastric cancer exhibit different patterns of genetic alterations. Pathologists in Europe and North America have designated gastric cancer histological type “intestinal type” and “diffuse type”, while pathologists in Japan have named these “well-differentiated type” and “poorly differentiated type”. The p53 mutation in tumor suppressor genes, cyclin E and p21 overexpression in cyclin and CDK inhibitors, TGF-α overexpression in growth factors, and CD44 aberrant transcript in cell adhesion molecules are often observed in both types of gastric cancer. In contrast, APC mutation and erbB-2 amplification are more likely to occur in well differentiated gastric cancers, and K-sam amplification and mutation or loss of E-cadherin are more likely to occur in poorly differentiated gastric cancers. Since specific carcinogens have specific mutagenic properties, the types of genetic mutation present in a specific form of gastric cancer may provide important clues to the mutagenic agents.

The results of epidemiological studies have suggested that *H. pylori* infection is closely linked to both types of gastric carcinoma. In addition, experiments using MG models have demonstrated that infection with *H. pylori* as well as administration of low-dose chemical carcinogens can induce both types of gastric carcinoma. Therefore, the best candidates for research on genetic alterations associated with *H. pylori* infection are p53 mutation, cyclin E and p21 overexpression, TGF-α overexpression, and CD44 aberrant transcript, which all preferentially occur in both types of gastric cancer.

The p53 is a nuclear protein and functions as a guardian of the genome. Wild type p53 binds to the responsive element on DNA and induces WAF1/CIP1/p21, which binds to cyclin-dependent kinase and induces G1 arrests. Another major function of p53 is to induce apoptosis via induction of Bax, Fas/Apo-1 and other apoptosis-related proteins. Therefore, mutant p53 might be linked to dysregulation of a cell cycle and an apoptosis. In fact, p53 mutation is the most widely described molecular alteration in human cancers. The mutation occurs in 40–70% of diffuse or intestinal type of human gastric cancer. Shiao et al. reported that p53 was mutated in 58% of patients with dysplasia and in 67% of patients with gastric cancer. Interestingly, Ochiai et al. reported that 4 of 10 incomplete type intestinal metaplasias demonstrated p53 mutation in exon 5 or exon 7 by PCR-SSCP analysis and direct sequencing. Therefore, p53 mutation might occur in the early steps of gastric carcinogenesis. As described in the previous part, MG is the best animal model for investigating gastric carcinogenesis induced by *H. pylori* infection, since this model closely resembles human pathology associated with *H. pylori* infection, chronic active gastritis, gastric atrophy, intestinal metaplasia and gastric carcinoma. However, since this animal has not been widely used in cancer experiments, there is, unfortunately, a lack of genetic information associated with oncogenesis, and there is also a lack of specific antibodies of MGs. These are disadvantages for progress of cancer study using this animal model. To overcome these bottlenecks, we need genetic information linked to oncogenesis in MGs, such as information on the p53 gene, which might be an appropriate target gene for investigating the oncogenic mechanisms of both types of gastric cancer induced by *H. pylori* infection. Therefore, we aimed to clone the p53 gene of the MG and to establish a yeast assay to detect functional mutation of p53 of the stomach infected by *H. pylori*. The complete sequence of the p53 cDNA of was deduced. The sequence was found to be 1173 bp in length and to encode 391 amino acids. The nucleotide sequence had a 78.8% homology to the human p53 cDNA. The amino acid sequence had a 76.2% homology to the human p53 protein. A novel assay system was established to detect functional mutations of the p53 gene in the stomach of the MGs using the nucleotide sequence (Fig. 1). To assess the background percentage of red colonies (mutant-type p53), liver, muscle, small intestine, brain, tongue, kidney, lung and stomach tissues of a specific pathogen free 5-week-old male MG were tested. All samples showed less than 10% red colonies and had no clonal mutations. To assess the quantification of the yeast functional assay, wild type and mutant-type p53 PCR products from normal liver tissue were mixed at serial ratios. The mixture of wild type and mutant-type p53 PCR products and the gapped pLSGP53 plasmid were co-transformed into yeast yIG397. A highly significant positive correla-
The p53 yeast functional assay: Total RNA extracted from tissues of Mongolian gerbil is amplified by RT-PCR and the PCR product is co-transformed into yeast with the gapped vector pLSGP53 carrying the 5′ and 3′ ends of p53 cDNA. Gap of the vector is repaired with the PCR product resulting in expression of p53 protein within yeast. Wild-type p53 protein binds to the p53 responsive element and activates transcription of the ADE2 gene, results in white colonies. Mutant-type p53 fails to express ADE2 and makes colonies red in a medium without leucine and with low concentration of adenine.

A positive correlation was observed between the percentage of red colonies and the content of mutant p53 cDNA \( (r^2 = 0.997) \). The main advantage of our system is that it enables evaluation of the transcriptional activity of the p53 protein. Another advantage of the yeast functional assay is that it enables quantitative analysis.

Watanabe et al. demonstrated the overexpression of p53 protein in the gastric cancer cells of MGs infected with H. pylori by using anti-human wild-type p53 antibody. Their results suggested that the p53 protein plays a role in H. pylori-induced gastric carcinogenesis in the gerbil. However, an immunohistochemical approach does not allow one to distinguish between wild type and mutant-type p53 proteins. For example, ultraviolet ray irradiation, gamma-irradiation and anticancer drugs induce DNA damage and result in an accumulation of wild-type p53 protein through a post-translational stabilization mechanism. Inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α), also induce an accumulation of wild-type p53 protein. In fact, TNF-α is produced in H. pylori-infected gastric mucosa in humans. Thus, an immunohistochemical method may detect an overexpression of wild-type p53 protein in the H. pylori-infected stomach. The p53 of MG displays only 76.2% homology to human p53, so it is uncertain whether anti-human p53 antibody will react with the p53 protein of the MG.

Correa provided a model of gastric carcinogenesis in which there is progression from active chronic gastritis, glandular atrophy, intestinal metaplasia and dysplasia resulting in gastric cancer. Alterations in the p53 gene are observed not only in cancer but also in the precancerous stage, especially in intestinal metaplasia in humans. As described previously, chronic H. pylori infection in the MG closely resembles that in humans. By using this animal model and the yeast p53 functional assay, it should be possible to elucidate when p53 gene alteration occurs in the process of gastric carcinogenesis in the H. pylori-infected stomach. For efficient prevention of gastric cancer by H. pylori eradication, the determination of the “point of no return” is required, that is, the reversible point of genetic alteration in a H. pylori-infected stomach. Sequential examination of the MG model after H. pylori infection may give important clues to verify the reversibility of p53 alteration using the yeast functional assay.

In vitro Epithelial Response infected with H. pylori

Another important approach for the gastric carcinogenesis induced by H. pylori infection is to investigate the molecular basis of epithelial cell response after H. pylori infection in vitro. In western epidemiological studies, the persons infected with cagA positive H. pylori strains had high risk for development of gastric cancers, compared with those cagA negative H. pylori strains. Although CagA protein is immunodominant, the function of CagA protein was not clearly elucidated. Cag pathogenicity island (CagPAI) including cagA gene functions as type 4 secretion machinery, which is a cylinder-like structure and a transfer system of H. pylori components to epithelial cells. In 1999, four groups reported the tyrosine on CagA protein was phosphorylated in epithelial cells and might trigger signal transduction of epithelial cells after the infection. Therefore, CagA protein is a key molecule to
investigate the interaction between the organism and the host. Recently, our group demonstrated that CagA protein was phosphorylated in epithelial cells after transfection of cagA gene and phosphorylated CagA protein formed a complex with SHP-2 type tyrosine phosphatase, which was a key molecule to induce a growth factor-like response in epithelial cells, via phosphorylated tyrosins on CagA protein. Interestingly, transfection of cagA gene induced a hummingbird morphology in epithelial cells and tyrosine phosphorylation and upregulation of SHP-2 type tyrosine phosphatase activity were essential for the induction of hummingbird morphology, which was similar morphology induced by treatment of hepatocyte growth factor (HGF) in epithelial cells. CagA protein, therefore, is responsible for dysregulation of intracellular signaling and cellular morphogenesis. These disruption of signal transduction system and morphology of epithelial cells after H. pylori infection may be associated with gastric carcinogenesis.

References


