mainly pro-tumor activity. In contrast, therapeutic interventions that enhance direct effector functions and indirect regulatory functions of neutophils result in potent anti-tumor activity. They postulate that conversion of pro-tumor activity of neutrophils into anti-tumor activity may offer a novel biological therapy of cancer. Drs. Masako Nakanishi and Daniel W. Rosenberg summarize the effects of a bioactive lipid, prostaglandin E2 (PGE_2), in gastrointestinal carcinogenesis and discussed the complex and interconnected pathways that link PGE_2 signaling with inflammation and cancer. Dr. Sergei Grivennikov reviews pathobiology of colitis-associated colorectal cancer in the large bowel. He introduced microbes as new players capable of triggering inflammation and possibly promoting carcinogenesis in the inflamed colon. Drs. Shmizu et al. postulate an association between obesity and hepatocellular carcinoma (HCC). They suggest multiple mechanisms, by which obesity influences HCC development, focusing on the emergence of insulin resistance and the subsequent activation of the inflammatory cascade. They also propose that nutritional or pharmaceutical approaches for preventing obesity-related liver carcinogenesis can be achieved by targeting pathophysiological conditions caused by obesity. Drs. Hiroko Oshima and Masanobu Oshima describe their own data regarding the role of PGE_2-induced inflammatory responses in gastric cancer development using their genetically altered mice, called as *Gan* mice. They demonstrate novel molecular pathways that are activated or suppressed in PGE_2-associated inflammatory microenvironment and thereby contribute to gastric cancer development. Dr. Mami Takahashi et al. describe pancreatic carcinogenesis, focusing on the roles of chronic inflammation and inflammatory factors in pancreatic carcinogenesis and the possible prevention of pancreatic ductal cancer using certain anti-inflammatory agents. They introduce interesting findings that a high-fat diet enhances hamster pancreatic carcinogenesis, along with aggravation of hyperlipidemia and severe fat infiltration (also observed in obese and diabetic patients) and increased expression of adipokines and inflammatory factors in the pancreas. Dr. Tsukamoto et al. report the role of *Helicobacter pylori* (*H. pylori*) infection in gastric carcinogenesis by using a novel experimental model, in which *H. pylori* infection induces intestinal metaplasia and intestinalization of stomach cancers independently. Finally, they mention that oxygen radical scavengers, anti-inflammatory chemicals, and the eradication of *H. pylori* are effective to prevent *H. pylori*-associated gastric carcinogenesis. Drs. Takuji Tanaka and Hideki Ishikawa present novel data on the role of mast cells in a mouse model of inflammation-associated colorectal carcinogenesis. They show that mice lacking mast cells are less susceptible to colitis-related colorectal tumorigenesis. Dr. Lee et al. review an association between inflammation and cancer development. They point out that inflammation (chronic inflammation) is a predisposing factor to carcinogenesis. They also emphasize that resolution of chronic inflammation is obtained by coordinated processes regulated by distinct anti-inflammatory and pro-resolving endogenous lipid mediators, including resolvins and lipoxins.

The aim of the guest editor is to provide a springboard to the ideas of new investigators currently devoted to cutting-edge research in these areas of inflammation and cancer. It exposes the reader to the exciting and fascinating cellular and molecular events that are involved in inflammation-associated carcinogenesis in a variety of tissues. I trust that the reader will share our enthusiasm and continued excitement for studying the cellular and molecular events in inflammation-associated carcinogenesis. Finally, we do hope that strategies to prevent or treat cancers will be developed in the near future based on insights into inflammatory pathways.

Anticancer effects of 4-vinyl-2,6-dimethoxyphenol (canolol) against SGC-7901 human gastric carcinoma cells

JING JIANG[1], DONG-HUI CAO[1], TETSUYA TSUKAMOTO[2], GUO-QING WANG[3], ZHI-FANG JIA[1], JIAN SUO[4] and XUE-YUAN CAO[4]

[1]Division of Clinical Epidemiology, First Hospital of Jilin University, Changchun, Jilin 130021, P.R. China;
[2]Division of Pathology, School of Medicine, Fujita Health University, Toyoake, Japan;
[3]Department of Pathogeny Biology, Norman Bethune Medical College of Jilin University;
[4]Department of Gastric and Colorectal Surgery, First Hospital of Jilin University, Changchun, Jilin 130021, P.R. China

Received December 12, 2012; Accepted February 27, 2013

DOI: 10.3892/ol.2013.1230

Abstract. Gastric cancer remains the fourth most commonly diagnosed cancer and is the second leading cause of cancer-related mortality worldwide. The aim of this study was to investigate the effects of canolol on the proliferation and apoptosis of SGC-7901 human gastric cancer cells and its relevant molecular mechanisms. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to observe the effect of canolol on the proliferation of SGC-7901 human gastric adenocarcinoma cells. The results showed that SGC-7901 cells exhibited a marked dose-dependent reduction in the proliferation rate. The survival rate of the cells was 88.86±1.58% at 50 μmol/l, decreasing to 53.73±1.51% at 800 μmol/l ($P<0.05$). By contrast, canolol had no significant toxicity on the human gastric mucosal epithelial cell line GES-1. The vivid images of cell morphology using an inverted microscope provided confirmation of the MTT assay. Treatment of SGC-7901 cells with canolol resulted in apoptosis demonstrated by flow cytometry. Furthermore, canolol downregulated the mRNA levels of COX-2, but had no significant effect on the mRNA expession of the Bax and Bcl-2 genes. These findings suggest that canolol has potential to be developed as a new natural anti-gastric carcinoma agent.

Introduction

Gastric cancer remains the fourth most commonly diagnosed cancer and is the second leading cause of cancer related mortality worldwide (1). Gastric cancer is the most common cancer in Eastern Asia (2). Eradication of *H. pylori* in the stomach by administration of oral antimicrobial agents results in the resolution of *H. pylori*-infected chronic active gastritis and significantly reduces the risk of gastric cancer development (3). However, bacterial eradication treatment has been lacking. The occurrence of antibiotic-resistant *H. pylori* has been reported (4) and is occasionally associated with adverse effects. Regular therapies such as chemotherapy, biotherapy and radiotherapy have been previously applied, however, they have unavoidable side effects (5). Therefore, more effective alternative approaches for gastric cancer prevention and therapies without undesirable side-effects are needed.

It is widely accepted that phytochemical, especially phenolic, compounds are associated with anticancer effects by affecting molecular events in the initiation, promotion and progression stages. Recent studies have demonstrated protective effects of plant phenolic compounds against gastric cancer (6-8). The expansion ability of tumor cells depends on the rate of both cell proliferation and cell apoptosis. The particular features of tumor cells allow them to evade apoptosis, a cell suicide program that reduce the damaged or mutated cells to maintain homeostasis (9).

Canolol, 4-vinyl-2,6-dimethoxyphenol (Fig. 1), is purified from crude canola oil and is a novel and potent antioxidant. Canolol has been proven to prevent *H. pylori*-induced gastritis and carcinogenesis in an animal model (10). However, its potential anti-proliferative and proapoptotic effects on gastric cancer cells and the possible mechanisms remain unknown.

The role of cyclooxygenase-2 (COX-2) inhibitors in the chemoprophylaxis of gastric cancer has been investigated. COX-2, the inducible isoform of COX, is undetectable in normal tissues and highly expressed in gastric tumors (11). Experimental studies have identified the correlation between COX-2 overexpression and the increased cell proliferation and decreased cell apoptosis in malignant tumor cells (12,13). COX inhibitors (Coxibs) are a series of drugs with analgesic, antipyretic and anti-inflammatory properties. Evidence suggests that COX-2 inhibitors correlate with tumor inhibition in breast (14) and endometrial cancer cell lines (15). Induction of apoptosis has increasingly become important with regard to the mechanism of cancer defense and prevention (16). However, the involvement of COX-2 inhibitors in gastric cancer prophy-

Correspondence to: Dr Xue-Yuan Cao, Department of Gastric and Colorectal Surgery, First Hospital of Jilin University, Changchun, Jilin 130021, P.R. China
E-mail: ccmzc32jdyycao@yahoo.com.cn

Key words: canolol, gastric cancer, COX-2, anti-proliferation, apoptosis

laxis remains to be determined, as the long-term use of COX-2 inhibitors exerts side-effects on the cardiovascular system and the digestive tract. A possible correlation between COX-2 inhibition and cell apoptosis in gastric cancer cell lines has yet to be examined.

In the present study, the effects of canolol on growth and apoptosis of human gastric adenocarcinoma SGC-7901 cells were investigated. Human gastric mucosal epithelial (GES-1) cells were used as the control cell model to examine the non-specific cytotoxicity of canolol. The mRNA expression levels of COX-2, Bcl-2 and Bax were detected to further elucidate the possible mechanisms involved.

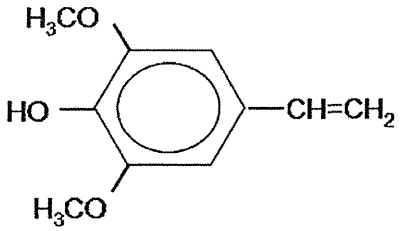

Figure 1. Chemical structure of canolol, 4-vinyl-2,6-dimethoxyphenol. Molecular weight: 180.

Materials and methods

Materials and reagents. 4-Vinyl-2,6-dimethoxyphenol (canolol with a molecular mass of 180) was purchased from Junsei Chemical, Tokyo, Japan. It was synthesized to at least 95% purity (confirmed by nuclear magnetic resonance). The preparation was sealed under helium or nitrogen and maintained at -80°C. Canolol was dissolved in ethanol and diluted in a serum-free medium immediately before the experiments. Gastric cancer SGC-7901 cells were obtained from the Department of Pathogeny Biology, Norman Bethune Medical College of Jilin University, China. Human gastric mucosal epithelial cell line GES-1 was obtained from the Cancer Hospital of Beijing University. The study protocol was approved by the ethics committee of the First Hospital of Jilin University.

Cell culture and treatment. Human SGC-7901 gastric cancer cell line and human GES-1 gastric mucosal epithelial cell line were cultured in RPMI-1640 medium containing 10% heat-inactivated fetal bovine serum (FBS) and 100 ng/ml each of penicillin and streptomycin in an incubator (50 ml/l CO_2) at 37°C. The medium was changed every 2-3 days. Cells in the logarithmic growth phase were collected for subsequent experiments. The cells were treated with various concentrations of canolol for 24 h.

Cell viability assay. The method of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was employed to determine cell viability. Cultured SGC-7901 and GES-1 cells were detached using trypsinization, centrifuged at 1,000 x g for 5 min and resuspended in fresh RPMI-1640 medium. The cells were plated at a density of $5x10^3$ cells/well in 96-well microplates and treated with canolol ranging from 25 to 1,200 μmol/l for 24 h at 37°C. At the end of treatment, 20 μl of MTT stock solution was added to each well [(0.5 mg/ml in phosphate-buffered saline (PBS)] for 4 h. The medium was replaced with 150 μl DMSO to dissolve the converted purple dye in the culture plates. Absorbance was measured at 570 nm on a spectrophotometer microplate reader. Cell viability was assayed as the relative formazan formation in treated compared with control wells after correction for background absorbance. Four wells per dose were counted in each experiment. Analyses were performed using SPSS version 10.0 (SPSS Inc, Chicago, IL, USA). Data were evaluated using one-way ANOVA. $P<0.05$ was considered statistically significant.

Cell morphology. SGC-7901 and GES-1 cells were seeded at a density of $5x10^5$ cells/well onto a cover slip loaded in 6-well plates. Fresh RPMI-1640 medium containing different concentrations of canolol was added. Cells were photographed with an inverted microscope under x200 magnifications to observe morphological changes.

Annexin V-FITC/PI staining for flow cytometry. SGC-7901 cells were collected and centrifuged at 1,000 x g for 5 min and resuspended in fresh RPMI-1640 medium at a density of $2x10^5$ cells/ml. Apoptotic and necrotic cells were evaluated by Annexin V (AV) binding and propidium iodide (PI) uptake using an AV-FITC-PI apoptosis assay kit (Pharmingen, San Diego, CA, USA). Samples were analyzed by flow cytometry.

Real-time quantitive PCR analysis. Total RNA of SGC-7901 cells was extracted using an RNA extraction kit and primers used are shown in Table I. Following DNase treatment, the first strand cDNA was synthesized. Quantitive PCR of Bcl-2, Bax and COX-2 were performed with the Bio-Rad (Hercules, CA, USA) CFX system. To exclude variations caused by RNA quantity and quality, the GAPDH gene was used as an internal control. Analyses were performed using SPSS version 10.0 (SPSS Inc). Data were evaluated using one-way ANOVA. $P<0.05$ was considered a statistically significant result.

Results

Canolol does not exhibit evident toxicity to GES-1 cells. The proliferation effect of canolol was determined using an MTT assay and GES-1 cells were used as a control to detect the cell toxicity of canolol. Cells were treated with different concentrations of canolol (0-1200 μmol/l). The data indicated that canolol has no obvious cytotoxicity against normal GES-1 cells. The percentage of cell viability was 99.38±3.57% at 25 μmol/l, 87.82±2.55% at 800 μmol/l and decreased to 65.31±4.44% at 1200 μmol/l (Fig. 2). Cell morphology using an inverted microscope also showed that cell structures were intact and were well established after 1,200 μmol/l canolol treatment (Fig. 3).

Canolol inhibits proliferation and induces apoptosis of SGC-7901 cells. SGC-7901 cells were treated with different concentrations of canolol (0-1200 μmol/l). The percentages of cell viability at various canolol doses were determined as the percentage of viable treated cells in comparison with

Table I. Primer sequences used in real-time quantitive PCR.

Gene	Primer sequence	Annealing temperature (°C)	Product size (bp)
COX-2	F: CTCCCTTGGGTGTCAAAGGTA R: GCCCTCGCTTATGATCTGTC	76	171
Bcl-2	F: GAGTTCGGTGGGGTCATG R: GGAGAAATCAAACAGAGGC	83	186
Bax	F: GGATGCGTCCACCAAGAA R: GAGCACTCCCGCCACAAA	83.5	388
GAPDH	F: AACGGATTTGGTCGTATTG R: GGAAGATGGTGATGGGATT	78.5	258

GAPDH, glyceraldehyde-3-phosphate dehydrogenase; F, forward; R, reverse.

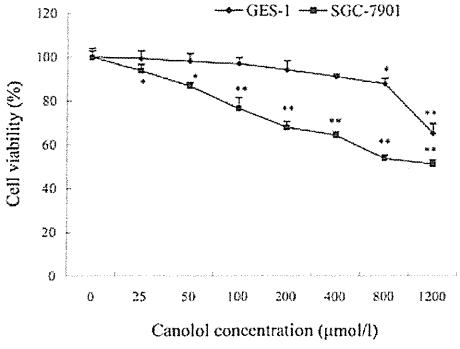

Figure 2. Effect of canolol on cell viability under different concentrations on particular cells using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (mean ± SD) (n = 4). Data were evaluated using one-way ANOVA. *P<0.05, **P<0.01.

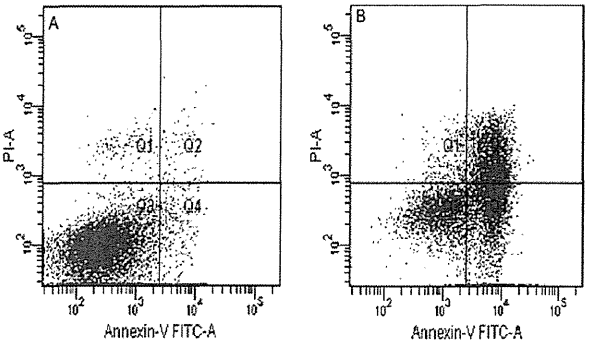

Figure 4. Apoptosis of SGC-7901 cells was investigated using a flow cytometry assay using FITC-Annexin-V/PI staining. (A) SGC-7901 cells without canolol; (B) SGC-7901 cells with 400 μmol/l canolol.

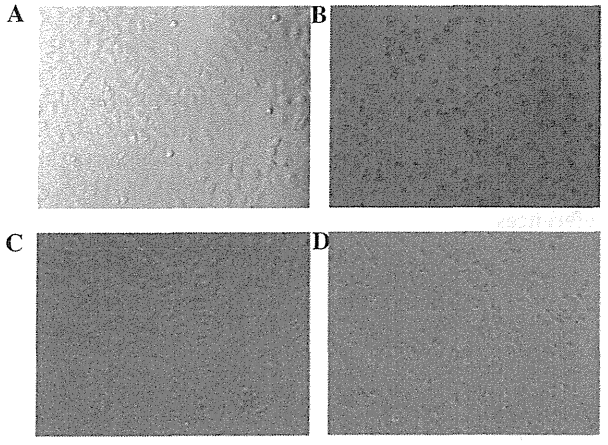

Figure 3. Morphology of GES-1 and SGC-7901 cells treated with 1,200 μmol/l canolol.

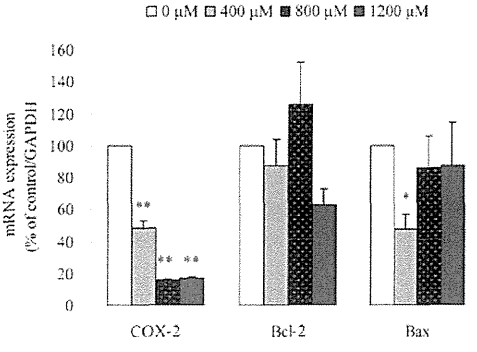

Figure 5. Relative expression levels of COX2, Bcl-2 and Bax mRNAs in SGC-7901 cells under treatment of different concentrations of canolol (mean ± SD) (n=3). Values are arbitrary unit values (mean ± SD) relative to 100 for controls. GAPDH was used as an internal control. Data were evaluated using one-way ANOVA. *P<0.05, **P<0.01 vs. control.

viable untreated cells. The results provided solid evidence that the inhibitory effects on the proliferation of canolol to SGC-7901 cells were dose-dependent (Fig. 2); the percentage of cell viability was 89.80±2.83% at 25 μmol/l, 73.73±1.51% at 800 μmol/l (P<0.05) and 51.22±1.82% at 1,200 μmol/l (P<0.01). Consistent with the MTT assay results, the adherent SGC-7901 cells were markedly decreased and showed apoptosis under the treatment of 1,200 μmol/l canolol (Fig. 3).

Furthermore, a flow cytometric analysis was used to quantify the rate of cell apoptosis using double staining of Annexin

V-FITC and PI. As shown in Fig. 4, the lower right field (high Annexin V, low PI staining) represents the early apoptotic cells due to the strong affinity of Annexin V-FITC with phosphatidylserine, which transports from the inner to the outer surface of the plasma membrane during early apoptosis. By contrast, the higher left field (high PI, low Annexin V staining) represents the necrotic cells, since PI, which binds to nucleic acids, only cross through the compromised membrane of dead cells or late apoptotic cells (17). Viable cells are shown in the lower left field (low Annexin V and PI staining) and the higher right field (high Annexin V and PI staining), indicating late apoptotic cells. The results showed that canolol was able to induce the apoptosis of SGC-7901 cells and the rate of early apoptosis, late apoptosis and necrosis of SGC-7901 cells were increased under 400 μmol/l canolol (Fig. 4).

Canolol downregulates the mRNA expression level of COX-2. To clarify the mechanisms of SGC-7901 cell apoptosis under canolol treatment, the mRNA expression level of COX-2, Bcl-2 and Bax was evaluated using real-time quantitive PCR. The sequences of these primers were shown in Table I. The results showed that in SGC-7901 cells, the relative mRNA expression level of COX-2 was decreased to 48.50±4.67% in 400 μmol/l, 16.08±0.75% in 800 μmol/l and 17.22±0.88% in 1,200 μmol/l canolol. The effect of canolol on COX-2 expression was downregulated ($P<0.01$); However, the expression levels of Bcl-2 and Bax fluctuated slightly (Fig. 5). These data suggested that the inhibition of COX-2 might play an important role in the apoptosis of SGC-7901 cells.

Discussion

Gastric cancer is one of the most prevalent malignant tumors and its morbidity is the highest in China. Currently, many natural and synthesized compounds are used in the chemoprovention and treatment of gastric cancer (18,19). Canolol, 4-vinyl-2,6-dimethoxyphenol, which is extracted from crude canola oil, has the ability to prevent *H. pylori*-infected gastric carcinogenesis in gerbils (10). In the present study, it was demonstrated that canolol prevented proliferation and induced apoptosis of SGC-7901 cells dose-dependently *in vitro*. Additionally, it had low toxicity to immortalized GES-1 cells (Figs. 2 and 3). The results indicated that canolol has the potential to be developed as a new natural anti-gastric carcinoma agent.

COX-2 is important in the conversion of arachidonic acid to prostaglandin H_2. Accumulating evidence suggests that the constitutive overexpresion of the inducible COX-2 gene is involved in a diverse array of cancers and Harris *et al* (20) demonstrated that COX-2 overexpresion initiated and promoted carcinogenesis through: i) mutagenesis, i.e., the production of certain reactive oxygen species that are carcinogenic; ii) mitogenesis, i.e., cell proliferation promoted by PGE-2 and other factors; iii) anti-apoptosis, i.e., cell differentiation and apoptosis reduced by PGE-2 and other factors; iv) angiogenesis, metastasis and immunosuppression (20). The real-time quantitive PCR in this study showed that COX-2 expression was downregulated under canolol treatment ($P<0.01$) (Fig. 5). It was postulated that inhibition of COX-2 expression may result in blockade of the prostaglandin cascade and a decrease in reactive oxygen species (ROS), thus stimulating apoptosis of malignant cells and preventing neoplastic growth. The scavenging potency of canolol against ROO$^{\bullet}$ is much higher than that of well-known antioxidants, such as α-tocopherol, vitamin C and β-carotene (21). A previous study in this laboratory showed canolol decreased serum 8-OHdG, a key biomarker of oxidative DNA damage relevant to carcinogenesis (10). Other natural phenolic extracts, such as BCE (black currant extract) and dioscin, reduce the risk of gastric cancer owing to their antioxidative functions (22-24).

Selective and non-selective COX-2 inhibitors may be involved in the intervention and chemoprevention of carcinogenesis (25-27). A series of epidemiologic studies found that the COX-2 inhibition levels of coxibs were consistent with their chemopreventive effects in cancers of the breast, colon, prostate and lung (20). Ma *et al* (28) have demonstrated that PGE2 acts with a family of G-protein-coupled receptors participating in multiple signal tranduction pathways.

The Bcl-2 family, such as Bax, Bad, Bid, Bcl-2 and Bcl-x, is one of the most extensively studied groups of proteins involved in cell apoptosis. Bax, Bad and Bid were shown to activate apoptosis, while Bcl-2 and Bcl-x were shown to inhibit the process (29). Transfection of COX-2 constitutive expression vector into the BCC cell line significantly upregulated Bcl-2 expression and this indicated that Bcl-2 might participate in COX-2 mediated anti-apoptic processes (30). In addition, the expression level of Bax, a member of a pro-apopotic protein family was downregulated in a transgenic mouse model (31). However, in the present study, no correlation between Bcl-2/Bax and COX-2 expression was found (Fig. 5).

The relationship between apoptosis and COX-2 downregulation in this gastric adenocarcinoma cells should be studied. COX-2 is a potential pharmacologic target that may be used in the prevention and treatment of various types of malignancies.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (Nos. 30972476 and 81273065). The authors would like to thank Dr Yu Chen for technical support.

References

1. Ferlay J, Shin H R, Bray F, *et al*: Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 127: 2893-2917, 2010.
2. Liu X, Zhang B, Guo Y, *et al*: Down-regulation of AP-4 inhibits proliferation, induces cell cycle arrest and promotes apoptosis in human gastric cancer cells. PLoS ONE 7: e37096, 2012.
3. Chuah SK, Tsay FW, Hsu PI and Wu DC: A new look at anti-*Helicobacter pylori* therapy. World J Gastroenterol 17: 3971-3975, 2011.
4. Graham D Y. Antibiotic resistance in *Helicobacter pylori*: implications for therapy. Gastroenterology 115: 1272-1277, 1998.
5. Siegel R, Naishadham D and Jemal A: Cancer statistics. CA Cancer J Clin 62: 10-29, 2012.
6. Kountouri AM, Kaliora AC, Koumbi L and Andrikopoulos NK: In-vitro gastric cancer prevention by a polyphenol-rich extract from olives through induction of apoptosis. Eur J Cancer Prev 18: 33-39, 2009.
7. Alvarez-Suarez JM, Dekanski D, Ristić S, *et al*: Strawberry polyphenols attenuate ethanol-induced gastric lesions in rats by activation of antioxidant enzymes and attenuation of MDA increase. PLoS One 6: e25878, 2011.

8. Jaganathan SK and Supriyanto E: Antiproliferative and molecular mechanism of eugenol-induced apoptosis in cancer cells. Molecules 17: 6290-6304, 2012.
9. Vaux DL and Korsmeyer SJ: Cell death in development. Cell 96: 245-254, 1999.
10. Cao XY, Tsukamoto T, Seki T, *et al*: 4-Vinyl-2,6-dimethoxyphenol (canolol) suppresses oxidative stress and gastric carcinogenesis in *Helicobacter pylori*-infected carcinogen-treated Mongolian gerbils. Int J Cancer 122: 1445-1454, 2008.
11. Harris RE, Chlebowski RT, Jackson RD, *et al*: Breast cancer and nonsteroidal anti-inflammatory drugs: prospective results from the Women's Health Initiative. Cancer Res 63: 6096-6101, 2003.
12. Kilic G, Gurates B, Garon J, *et al*: Expression of cyclooxygenase-2 in endometrial adenocarcinoma. Eur J Gynaecol Oncol 26: 271-274, 2005.
13. Targosz A, Brzozowski T, Pierzchalski P, Szczyrk U, Ptak-Belowska A, Konturek SJ and Pawlik W: *Helicobacter pylori* promotes apoptosis, activates cyclooxygenase (COX)-2 and inhibits heat shock protein HSP70 in gastric cancer epithelial cells. Inflamm Res 61: 955-966, 2012.
14. Ashok V, Dash C, Rohan TE, Sprafka JM and Terry PD: Selective cyclooxygenase-2 (COX-2) inhibitors and breast cancer risk. Breast 20: 66-70, 2011.
15. Ozalp SS, Eren CY, Bostancioğlum RB and Koparal AT: Induction of apoptosis and inhibition of cell proliferation by the cyclooxygenase enzyme blocker-nimesulide in the Ishikawa endometrial cancer cell line. Eur J Obstet Gyn Reprod Biol 164: 79-84, 2012.
16. Sun SY, Hail JRN and Lotan R: Apoptosis as a novel target for cancer chemoprevention. J Natl Cancer Inst 96: 662-672, 2004.
17. Tripathi M, Singh BK, Mishra C, Raisuddin S and Kakkar P: Involvement of mitochondria mediated pathways in hepatoprotection conferred by *Fumaria parviflora Lam*. Extract against nimesulide induced apoptosis in vitro. Toxicol In Vitro 24: 495-508, 2010.
18. Wu XY, Wang YL, Wang HW, *et al*: Quinacrine inhibits cell growth and induces apoptosis in human gastric cancer cell line SGC-7901. Cur Ther Res Clin Exp 73: 52-64, 2012.
19. Ji YB, Qu ZY and Zou X: Juglone-induced apoptosis in human gastric cancer SGC-7901 cells via the mitochondrial pathway. Exp Toxicol Pathol 63: 69-78 2011.
20. Harris RE, Beebe J and Alshafie GA: Reduction in cancer risk by selective and nonselective cyclooxygenase-2 (COX-2) inhibitors. J Exp Pharmacol 6: 491-496, 2012.
21. Dong X, Li ZR, Wang W, Zhang WJ, Liu SZ and Zhang XM: Protective effect of canolol from oxidative stress-induced cell damage in ARPE-19 cells via an ERK mediated antioxidative pathway. Mol Vis 17: 2040-2048, 2011.
22. Jia N, Xiong YLL, Kong BH, Liu Q and Xia XF: Radical scavenging activity of black currant (*Ribes nigrum L.*) extract and its inhibitory effect on gastric cancer cell proliferation via induction of apoptosis. J Funct Foods 4: 382-390, 2011.
23. Gao LL, Li FR, Jiao P, *et al*: Paris chinensis dioscin induces G2/M cell cycle arrest and apoptosis in human gastric cancer SGC-7901 cells. World J Gastroenterol 17: 4389-4395, 2011.
24. Hu MM, Xu LN, Yin LH, *et al*: Cytotoxicity of dioscin in human gastric carcinoma cells through death receptor and mitochondrial pathways. J Appl Toxicol doi: 10.1002/jat.2715, 2012.
25. Fu SL, Wu YL, Zhang YP, Qiao MM and Chen Y: Anti-cancer effects of COX-2 inhibitors and their correlation with angiogenesis and invasion in gastric cancer. World J Gastroenterol 10: 1971-1974, 2004.
26. Harris RE: Cyclooxygenase-2 (COX-2) blockade in the chemoprevention of cancers of the colon, breast, prostate, and lung. Inflammopharmacology 17: 55-67, 2009.
27. Chan AT, Ogino S and Fuchs CS: Aspirin use and survival after diagnosis of colorectal cancer. JAMA 302: 649-658, 2010.
28. Ma X, Kundu N, Rifat S, Walser T and Fulton AM: Prostaglandin E receptor EP4 antagonism inhibits breast cancer metastasis. Cancer Res 66: 2923-2927, 2006.
29. Tsujimoto Y and Shimizu S: Bcl-2 family: life-or-death switch. FEBS Lett 466: 6-10, 2000.
30. Tjiu JW, Liao YH, Lin SJ, *et al*: Cyclooxygenase-2 overexpression in human basal cell carcinoma cell line increases antiapoptosis, angiogenesis, and tumorigenesis. J Invest Dermatol 126: 1143-1151, 2006.
31. Liu CH, Chang SH, Narko K, *et al*: Overexpression of cyclooxygenase-2 is sufficient to induce tumorigenesis in transgenic mice. J Biol Chem 276: 18563-18569, 2001.

Semin Immunopathol (2013) 35:177–190
DOI 10.1007/s00281-012-0357-1

REVIEW

Helicobacter pylori infection and gastric carcinogenesis in rodent models

Tetsuya Tsukamoto · Takeshi Toyoda · Tsutomu Mizoshita · Masae Tatematsu

Received: 24 July 2012 / Accepted: 15 October 2012 / Published online: 31 October 2012

Abstract *Helicobacter pylori* infection is an important factor for gastric carcinogenesis in human. In carcinogen-treated Mongolian gerbils, *H. pylori* infection enhances stomach carcinogenesis, while infection alone induced severe hyperplasia called heterotopic proliferative glands. A high-salt diet or early acquisition of the bacteria exacerbates inflammation and carcinogenesis. Oxygen radical scavengers or anti-inflammatory chemicals as well as eradication of *H. pylori* are effective to prevent carcinogenesis. *H. pylori*-associated inflammation induces intestinal metaplasia and intestinalization of stomach cancers independently. It is necessary to control cancer development not only in *H. pylori*-positive cases but also in *H. pylori*-negative metaplastic gastritis.

Keywords *Helicobacter pylori* · Mongolian gerbil · Intestinal metaplasia · Gastric adenocarcinoma · Chemoprevention

This article is a contribution to the special issue on Inflammation and Cancer - Guest Editor: Takuji Tanaka

T. Tsukamoto (✉)
Department of Diagnostic Pathology, Fujita Health University School of Medicine,
1-98 Dengakugakubo, Kutsukake-cho,
Toyoake, Aichi 470-1192, Japan
e-mail: ttsukamt@fujita-hu.ac.jp

T. Tsukamoto
e-mail: ttsukamt@gmail.com

T. Tsukamoto
Department of Pathology and Matrix Biology, Mie University Graduate School of Medicine,
2-174 Edobashi,
Tsu, Mie 514-8507, Japan

T. Toyoda
Division of Pathology, National Institute of Health Sciences,
1-18-1 Kamiyoga,
Setagaya-ku, Tokyo 158-8501, Japan

T. Mizoshita
Department of Internal Medicine and Bioregulation, Nagoya City University Graduate School of Medical Science,
1-Kawasumi, Mizuho-cho,
Mizuho-ku, Nagoya 467-8601, Japan

M. Tatematsu
Japan Bioassay Research Center,
2445 Hirasawa,
Hadano, Kanagawa 257-0015, Japan

Introduction

Gastric cancer is in decreasing trend nowadays [1 19], however, it remains the fourth most common cancer and second leading cause of cancer-related death worldwide [76]. It is still an important medical problem and its prevention is one of the most important aspects of cancer control programs. Many pathological and biological analyses of gastric carcinomas, including precancerous lesions, have been performed with experimental animals as well as human samples. Major model animals susceptible to induction of gastric adenocarcinomas include rats, mice, and Mongolian gerbils, the latter two offering powerful tools for analysis of *Helicobacter pylori*-associated gastric disorders. In this article, we introduce major animal models while comparing with human lesions, concentrating special attention on pathological and biological findings.

H. pylori infection and the development of gastric cancer: epidemiological findings

H. pylori has been discovered from patients of chronic gastritis as gram-negative, flagellate, and microaerophilic

bacilli [52, 127] and has been revealed as a major causative factor for gastric disorders. Strong epidemiological evidence has been accumulated indicating a significant relationship with active chronic gastritis, peptic ulcers, atrophic gastritis, intestinal metaplasia, and malignant lymphoma or cancer development [3, 15, 18, 23, 28, 29, 42, 67, 77, 78, 114]. Prospective study confirmed that gastric cancers developed in 2.9 % of the *H. pylori*-infected group but none of the uninfected patients [126]. Based on the epidemiological findings, *H. pylori* was defined as a "definite biological carcinogen" by World Health Organization/International Agency for Research on Cancer (WHO/IARC) in 1994. However, regarding the relationship between *H. pylori* infection and development of gastric cancers, the lack of evidence of induction in experimental animals was one point, which received stress in the WHO/IARC report [30].

Establishment of the animal models

To identify pathological and molecular biochemical mechanisms, various experimental animal models have been established in rats and mice with chemical carcinogens including *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) and *N*-methyl-*N*-nitrosourea (MNU). Since *H. pylori* is one of the most important factors for human stomach disorders, the *H. pylori*-infected and carcinogen-treated Mongolian gerbil has proven very useful for analyses of underlying processes [123].

Rat models

In some of the earliest studies, researchers attempted to induce gastric cancers in animal models using chemical carcinogens such as benzo[a]pyrene [82] or 3-methylcholanthrene [90] by direct injection into the stomach or the gastric wall. Others tried oral administration, a more natural administrative method, using 2-acethylaminofluorene [129]. However, the resultant incidences of lesions were low. In 1967, however, Sugimura and Fujimura [92] utilized *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) in drinking water and were able to report good yields of adenocarcinomas in the glandular stomachs of rats. Upon exposure to MNNG, erosive lesions occurred and subsequent disordering of glandular structures and proliferation of epithelial cells in the pyloric mucosa were observed. After appearance of atypical glands, finally both differentiated and undifferentiated adenocarcinomas were induced in this model, mimicking the situation in humans. Oral administration of 4-nitroquinoline 1-oxide (4-NQO) and 4-hydroxyaminoquinoline 1-oxide (4-HAQO) similarly induces carcinomas in the stomach as well as the various other tissues [61, 62]. The presence of surfactants, such as alkylbenzenesulfonate, was found to enhance the effects of 4-NQO in the stomach of animals [96, 99]. Another carcinogen, *N*-ethyl-*N'*-nitro-*N*-nitrosoguanidine [43, 94], could be shown to cause gastric cancers not only in rats but also in dogs.

Mouse models

Administration of MNNG in the drinking water to BRSUNT/NJms mice over the life span only resulted in adenomatous hyperplasia of the gastric epithelium, suggesting resistance to this carcinogen [93]. However, oral administration of 4-NQO and 4-HAQO did induce carcinomas in the stomach as well as various other tissues [61, 62]. Finally, MNU was successfully introduced for good yields of adenocarcinomas in the glandular stomach of BALB/c mice [110]. Comparable yields were obtained in the C_3H [111] and other strains [133]. In the mouse models, both differentiated and undifferentiated types of adenocarcinomas typically develop, showing more significant cellular atypia compared with lesions in MNNG-treated rats. Taking advantage of this characteristic, intramucosal neoplasms became recognizable. The establishment of mouse models further opened up new approaches using transgenic and knockout animals. Yamamoto et al. utilized p53 knockout mice and revealed that nullizygous p53 knockout mice showed higher susceptibility to carcinogen in contrast to heterozygous animals [132].

Helicobacter-infected mouse models

H. pylori infection has been tried on many animals to study the pathogenetic background, but none of the early models proved sufficiently similar to the human situation [41, 80]. In 1990, Lee et al. [46] isolated *Helicobacter felis* from the cat stomach and inoculated germ-free mice with the bacteria, which colonized in the stomach like as *H. pylori* and induced acute and chronic inflammation. p53 wild-type and hemizygous mice were inoculated with *H. felis* and the latter showed higher proliferative index in the gastric foveolar epithelium [19]. *H. pylori* isolated from human clinical specimens were also utilized to inoculated nude and euthymic mice causing chronic active gastritis [34, 35, 51]. *H. pylori*, named Sydney strain (SS1), with higher colonizing ability were established by screening of fresh clinical isolates in long-term mouse adaptation and is currently widely used worldwide for mice experiments [47].

Oshima et al. constructed transgenic mice (K19-C2mE transgenic mouse) simultaneously expressing cyclooxygenase-2 (COX-2) and microsomal prostaglandin E synthase-1 under keratin 19 promoter in the gastric epithelial cells and revealed the importance of these pathways in gastric tumorigenesis especially with *Helicobacter* infection [72]. Additional expression of Wnt1 converted more dysplastic gastric tumors [73]. MNU treatment and *H. pylori* (Sydney strain, SS1) infection of K19-C2mE mice induced adenocarcinomas

not only in pyloric mucosa but also in fundic glands, serving a better model for increasing proximal gastric cancers [101].

H. pylori-infected Mongolian gerbil models: promoting effects of *H. pylori*

Besides the mouse models, Mongolian gerbil (*Meriones unguiculatus*) model was successfully established to mimic human severe *H. pylori* infection and inflammation, with the bacteria detectable throughout the study period up to 1 year [25]. Gerbils can be readily infected with *H. pylori*, and the resultant chronic active gastritis, peptic ulcers, and intestinal metaplasia resemble lesions apparent in man (Fig. 1a). Later in 1998, stomach carcinogenesis model was established using Mongolian gerbils with MNU and MNNG as the carcinogens (Fig. 1b) [112]. *H. pylori* infection was subsequently found to increase the incidence of both MNU- and MNNG- induced adenocarcinomas of all histological types including differentiated and undifferentiated adenocarcinomas and signet-ring cell carcinoma in the gerbils' glandular stomach (Fig. 1c, d) [85, 86, 95]. Several studies based on detailed histopathological assessment showed no carcinomas in animals treated only with *H. pylori* infection [85–87, 95, 112]. Thus, we consider that *H. pylori* is a strong promoter of gastric carcinogenesis. The *H. pylori*-infected and chemical carcinogen-treated Mongolian gerbils have thus proved very useful for the analysis of gastric carcinogenesis.

Besides adenocarcinomas, neuroendocrine tumors (NET) (endocrine cell hyperplasia/dysplasia and carcinoid tumors) were frequently induced in *H. pylori*-infected Mongolian gerbil model in association with serum gastrin level. Eradication of *H. pylori* prevents occurrence of NET lesions in the glandular stomach, this being strongly linked with reduction in serum gastrin levels [9]. Proton pump inhibitors (PPIs), routinely used for control of upper gastrointestinal disorders, have been some concern about the long-term safety and the possibility of cancer induction and development of NET in the stomach. When PPI was administered to *H. pylori*-infected Mongolian gerbils, PPI at high dose increased NET development with higher serum gastrin; in contrast, PPI at low dose had no influence on development of carcinomas and NETs in the *H. pylori*-infected and uninfected gerbils' glandular stomach [124].

Modifying factors of stomach carcinogenesis

Host risk factors for gastric cancer: age of infection of *H. pylori*

Childhood infection with *H. pylori* is a major concern in the pediatrics field. To compare severity of inflammation and susceptibility to induction of cancer in childhood and the adult, *H. pylori* inoculation and subsequent MNU administration were started at different time points in the gerbil's lifespan. Early acquisition of *H. pylori* significantly increased gastric carcinogenesis in carcinogen-treated gerbils, as compared to the case with later infection, possibly because of differences in host gastric mucosal and immunologic factors, as well as age-dependent sensitivity to chemical carcinogens (Fig. 1e, f) [5]. This would imply that childhood *H. pylori* infection must not be overlooked in

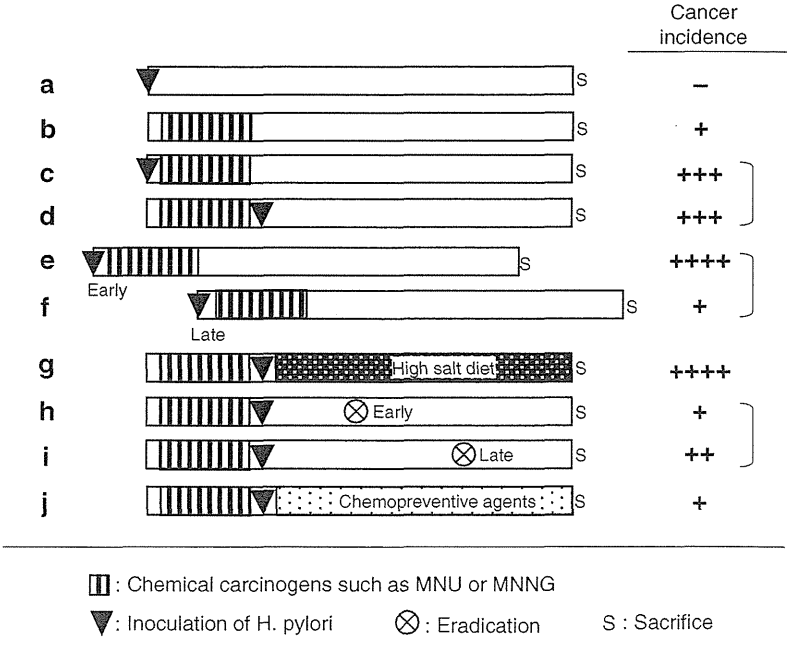

Fig. 1 Modifying factors for *H. pylori*-associated stomach carcinogenesis in Mongolian gerbils. **a** *H. pylori* infection alone is not carcinogenic. **b** Drinking water containing chemical carcinogens including MNU or MNNG induces stomach cancers. **c**, **d** When combined, *H. pylori* become a strong promoter. **e**, **f** Earlier infection increases the risk than later event. **g** A high-salt diet exacerbates inflammation and increases the incidence of *H. pylori*-associated cancer. **h**, **i** Earlier eradication of *H. pylori* reduces risk of stomach cancers. **j** Various natural products and pure chemicals appear to have chemopreventive potential

approaches to the prevention of gastric cancer later in adult life [14, 50].

Synergistic effects of *H. pylori* and high-salt diet

Among various food ingredients, salt and salted foods are probable risk factors for gastric cancer, based on evidence from a large number of case–control and ecological studies [33, 39, 97, 118]. In an experimental study, sodium chloride (NaCl) was found to enhance the carcinogenic effects of MNNG and 4-NQO in the rat glandular stomach [103], possibly partly due to decrease in the viscosity of the gastric mucus and so reduction in the protective mucous barrier. When given alone, NaCl has no apparent carcinogenicity in rats but, when administered with MNNG or 4-NQO, it promotes gastric carcinogenesis in the rat glandular stomach [103], in a dose-dependent fashion [100]. A high concentration causes initial tissue damage and consequent regenerative cell proliferation and this is also in line with accelerated lesion development [21].

Furthermore, in 2002, Nozaki et al. demonstrated a high-salt diet to enhance the effects of *H. pylori* infection on gastric carcinogenesis, with these two factors acting synergistically to promote the development of stomach cancers in the Mongolian gerbil model, although high-salt intake alone has a minor influence compared to *H. pylori* [68]. Further, to examine the dose dependence and the mechanisms underlying enhancing effects, Mongolian gerbils were treated with MNU, *H. pylori*, and food containing various concentrations of salt, and were sacrificed after 50 weeks. Among gerbils treated with MNU and *H. pylori*, the incidences of glandular stomach cancers were 15 % in the normal diet group and 33, 36, and 63 % in the 2.5, 5, and 10 % NaCl diet groups, showing dose-dependent increase. Intermittent intragastric injection of saturated NaCl solution, in contrast, did not promote gastric carcinogenesis. In gerbils infected with *H. pylori*, a high-salt diet was associated with elevation of anti-*H. pylori* antibody titers, serum gastrin levels, and inflammatory cell infiltration in a dose-dependent fashion (Fig. 1g). Ten percent NaCl diet upregulated the amount of surface mucous cell mucin, suitable for *H. pylori* colonization, despite no increment of MUC5AC mRNA, while *H. pylori* infection itself had an opposing effect, stimulating transcription of MUC6 and increasing the amount of gland mucous cell mucin. High-salt diet, in turn, decreased the amount of gland mucous cell mucin, which acts against *H. pylori* infection by inhibiting the bacterial cell wall component [38]. Reduction of salt intake could thus be one of the most important chemopreventive methods for human gastric carcinogenesis [36].

Besides the salt itself, *H. pylori* infection and high intake of various traditional salt-preserved foods are regarded as risk factors for human gastric cancer. Indole compounds, such as indole-3-acetonitrile is contained in Chinese cabbage and converted to a mutagen, 1-nitrosoindole-3-acetonitrile (NIAN), with nitrite under acidic condition in the stomach. Administration of NIAN to Mongolian gerbils induced well to moderately differentiated adenocarcinomas under *H. pylori* infection. Such lesions were not induced in gerbils given NIAN alone or infection with *H. pylori* alone. Thus, endogenous carcinogens formed from nitrosation of indole compounds could be critical risk factors for human gastric cancer development under the influence of *H. pylori* infection. [53]

Prevention of gastric cancer by eradication of *H. pylori*

To clarify the effects of eradication of *H. pylori* on prevention of gastric cancer development in patients with chronic gastritis, Uemura et al. [125] conducted a nonrandomized *H. pylori* eradication trial in cases whose gastric cancer was removed by endoscopic resection, and suggested that *H. pylori* eradication might improve neutrophil infiltration and intestinal metaplasia in the gastric mucosa and inhibit the development of new carcinomas. A randomized controlled trial conducted in China revealed that the incidence of gastric cancer development at the population level was similar between participants receiving *H. pylori* eradication treatment and those receiving placebo over a period of 7.5 years [130]. However, in the subgroup of *H. pylori* carriers without precancerous lesions, eradication of *H. pylori* significantly decreased the development of gastric cancer [130]. In *H. pylori*-infected Mongolian gerbils treated with MNU, Shimizu et al. have provided direct evidence that *H. pylori* eradication may be useful as a prevention approach against gastric cancer [87]. The incidences of gastric cancers after curative treatment for *H. pylori* were thus significantly lower than without *H. pylori* eradication. For further evaluation, an experimental model with eradication in the early, middle, and late periods was studied using *H. pylori*-infected and MNU-treated Mongolian gerbils [70]. *H. pylori* infection was found to strongly enhance gastric carcinogenesis initiated with the chemical carcinogen, and following eradication at an early period, this effect was effectively reduced (Fig. 1h, i).

Reversibility of heterotopic proliferative glands induced by *H. pylori* infection

As WHO/IARC has mentioned in 1994 [30], it had to be clarified whether or not *H. pylori* itself was a carcinogen. Several studies based on histopathology showed no carcinomas in animals treated only with *H. pylori* infection [85–87, 95, 112]. However, two reports concluded that *H. pylori* infection alone can induce well-differentiated adenocarcinomas at very high incidences in the glandular stomach

of Mongolian gerbils [27, 128], while another study resulted in only one poorly differentiated adenocarcinoma [26]. The incidences and histological patterns of the lesions differed greatly in these three reports. After *H. pylori* infection, glands in the stomach of gerbils start to proliferate into the submucosa, disrupting the lamina muscularis mucosa. Resultant lesions, termed heterotopic proliferative glands (HPGs), frequently develop with *H. pylori* infection in the glandular stomach of infected Mongolian gerbils, with minimal dysplastic change of constituent cells [69]. HPGs often resemble differentiated or mucinous adenocarcinomas showing structural abnormality, but lacked obvious cellular atypia. Their characteristics are: (1) organized polarity of their component cells; (2) differentiation from gastric phenotype HPGs into intestinal phenotype HPGs with mature Paneth cells; (3) formation of large cystic dilatations containing mucin, often with calcification; (4) shedding of epithelial cells and necrosis at the tips of lesions; (5) high-grade inflammation with infiltration of inflammatory cells (neutrophils in acute phase and mononuclear cells in chronic phase); and (6) organized polarity of proliferating zones (Table 1 and Fig. 2) [69]. These features are quite different from those of well-differentiated adenocarcinomas, which are characterized by obvious cellular atypia. After eradication, HPGs are obviously reduced, and gastric lesions in mucosa also disappear with little evidence persisting of the former injury. Reversible HPGs are induced solely by *H. pylori* infection in this species, and our studies have shown they are related to severe gastritis, rather than being malignant in character. Eradication of *H. pylori* induces apoptosis and suppresses proliferation in HPGs of infected Mongolian gerbils, these lesions thus being apparently reversible through regulation of cell kinetics [6]. Thus, distinguishing reversible lesions from true neoplasms is necessary in investigating the relationship of *H. pylori* infection with gastric carcinogenesis in the Mongolian gerbil model [69]. Taking into account all the available data, we conclude that *H. pylori* is a strong promoter of gastric carcinogenesis rather than an initiator.

Chemoprevention of gastric cancer

COX-2 inhibitor

Overexpression of COX-2 has been shown to be associated with several cancers, including gastric and colorectal adenocarcinomas in mice models [48, 72, 73, 79, 101] (Fig. 1j). Furthermore, COX-2 selective inhibitors such as etodolac and celecoxib may have chemopreventive effects [22, 49] not only suppressing inflammation, but also causing regression of early-stage tumors [10, 81]. Therefore, there is a possibility that COX-2 inhibitors could be useful drugs for regression of remaining precancerous lesion and prevention of gastric cancer occurrence after *H. pylori* eradication.

Considering the human situation, eradication of *H. pylori* significantly lowers the development of metachronous gastric cancer [20]. However, there has not been any approach to prevent gastric carcinogenesis in extensive metaplastic gastritis, an *H. pylori*-negative precancerous lesion. Forty-seven patients with extensive metaplastic gastritis were selected based on endoscopic findings and serum pepsinogen test-positive and *H. pylori* antibody-negative conditions. Nonrandomized etodolac treatment (300 mg/day) was administered to 26 patients, while the remaining 21 were untreated up to 5 years. Five cancers developed in non-treated group significantly more than one case in the etodolac group. Long-term etodolac treatment effectively reduced metachronous cancer development in patients with extensive metaplastic gastritis, while it did not influence the extent of metaplastic gastritis. Regulation of COX-2 could be an effective chemoprevention of gastric cancer in the metaplastic gastritis. [134]

Table 1 Histological difference of heterotopic proliferative glands and well-differentiated adenocarcinoma

	Heterotopic proliferative glands (HPGS)	Well-differentiated adenocarcinoma
Distribution of glands	Dispersed	Compactly proliferated
Shape of glands	Large and cystic	Relatively small
Intracystic material	Eosinophilic, sometimes with calcification	Usually transparent
Lining epithelium	Shedding of epithelial cells with necrosis at the tip of the cyst	Fully lined with atypical cells.
Intestinalization	Frequent, sometimes with Paneth cells	Relatively infrequent
Stroma	Severe inflammatory cell infiltrates. Neutrophils in acute phase. Lymphocytes and plasma cell in chronic phase with lymphoid follicle formation.	Desmoplastic reaction
Cellular component	No atypia. Organized polarity. Partly degenerated.	Enlarged nuclei with increased chromatin. Loss of polarity.

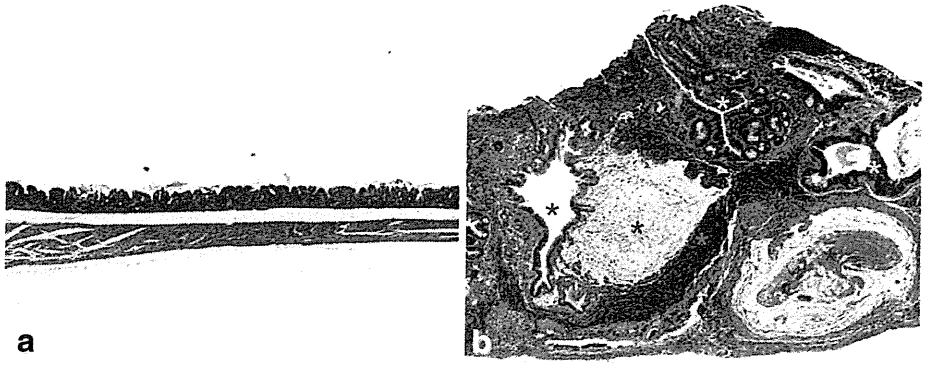

Fig. 2 Severe inflammatory response caused by long-term *H. pylori* infection in a Mongolian gerbil. **a** Normal gastric mucosa without *H. pylori* infection. **b** Heterotopic proliferative glands (HPGs) in the glandular stomach infected with *H. pylori* for 75 weeks. Proliferation and dilatation of gastric type gland (*red asterisk*) and intestinal metaplastic glands (*yellow asterisk*) with lymphoid follicle formation (*green asterisk*). Mucous lakes (*blue asterisks*) are developed with necrotic material (*blue asterisk on the right*). Hematoxylin and eosin staining

Oxygen radical scavenger

Recent epidemiological and experimental studies have demonstrated that consumption of certain natural products can lower gastric cancer risk in humans and animal models [120]. It has been also suggested that oxidative stress associated with inflammation plays an important role in gastric carcinogenesis as a mediator of DNA damage and carcinogenic compound formation [64]. Since the major determinant factor of gastric carcinogenesis is the severity of *H. pylori*-induced inflammation [7], the inhibition of *H. pylori*-induced inflammation and subsequent oxidative stress is a reasonable approach to prevent gastric cancer development.

To assess this hypothesis in the gerbil model, 4-vinyl-2,6-dimethoxyphenol (canolol), one of the most potent antioxidative compounds obtained from crude canola oil, was chosen to examine preventive effect of *H. pylori*-induced gastritis and gastric carcinogenesis [8]. The gerbils were subjected to *H. pylori*+MNU administration and were fed for 44 weeks with or without 0.1 % canolol. *H. pylori*-induced gastritis, expression of COX-2 and inducible nitric oxide synthase (iNOS), and increase in serum 8-hydroxy-2′-deoxyguanosine (8-OHdG) level were all attenuated in the canolol-treated groups. In addition, the incidence of gastric adenocarcinomas was markedly reduced. These data indicate that oxygen radical scavengers may suppress gastric inflammation and carcinogenesis in *H. pylori*-infected gerbils. Interestingly, the viable *H. pylori* count was not changed by the canolol-containing diet. Thus, the data point to the level of inflammation due to *H. pylori* rather than the existence of the bacteria as the determining factor is used.

Lignan, a plant-derived chemical

Another example of a chemopreventive agent is plant-derived lignan, a major group of plant compounds classified as phytoestrogens, which have attracted interest in recent years [1]. (+)-Syringarecinol, one of lignans contained in Japanese apricot, inhibited >90 % of the *H. pylori* motility at a concentration of 500 μg/mL and the IC50 value was 50 μg/mL *in vitro* [55]. Concentrated Japanese apricot significantly alleviated *H. pylori*-induced inflammation in Mongolian gerbils [75].

Since other lignans including arctiin, arctigenin, and nordihydroguaiaretic acid (NDGA) also inhibited proliferation and motility of *H. pylori in vitro* dose dependently, *in vivo* analyses were performed to assess preventive effect on *H. pylori*-associated gastritis and gastric cancer development in Mongolian gerbils using NDGA and arctigenin [115]. NDGA significantly decreased the incidence of gastric adenocarcinomas, the formation of intestinal metaplasia, and serum 8-OHdG levels. There were no differences in the titers of anti-*H. pylori* IgG or the expression of the *H. pylori*-specific *urease A* gene among all *H. pylori*-infected groups. These results suggest that NDGA might have suppressive effects on gastric carcinogenesis, with inhibitory effects on progression of gastritis and antioxidative activity rather than direct antimicrobial influence as the major mechanisms. Arctigenin, in contrast, failed to attenuate neoplasia in gerbils in spite of its potent suppressive effect on *H. pylori in vitro*. These results suggest the importance of *in vivo* animal experiments as well as *in vitro* analyses.

Nuclear factor-κB inhibitor

Nuclear factor-κB (NF-κB) plays a major role in host inflammatory responses and carcinogenesis and as such is an important drug target for adjuvant therapy. One of NF-κB inhibitors, caffeic acid phenethyl ester (CAPE), was analyzed on *H. pylori*-induced NF-κB activation in cell culture and chronic gastritis in Mongolian gerbils. In AGS human gastric cancer cells, CAPE significantly inhibited *H. pylori*-

stimulated NF-κB activation and mRNA expression of several inflammatory factors in a dose-dependent manner, and prevented degradation of IκB-α and phosphorylation of p65 subunit. To evaluate the effects of CAPE on *H. pylori*-induced gastritis, Mongolian gerbils were inoculated with *H. pylori*, fed diets containing 0.1 % CAPE, and sacrificed after 12 weeks. Infiltration of neutrophils and mononuclear cells and expression of NF-κB p50 subunit and phospho-IκB-α were significantly suppressed by CAPE treatment in the antrum of *H. pylori*-infected gerbils. Labeling indices for 5′-bromo-2′-deoxyuridine both in the antrum and corpus were markedly reduced at the highest dose, suggesting a preventive effect of CAPE on epithelial proliferation. Furthermore, in the pyloric mucosa, mRNA expression of inflammatory mediators including tumor necrosis factor-α (TNFα), interferon-γ, interleukin (IL)-2, IL-6, KC (IL-8 homologue), and iNOS was significantly reduced. These results suggest that CAPE has inhibitory effects on *H. pylori*-induced gastritis in Mongolian gerbils through the suppression of NF-κB activation, and may thus have potential for prevention and therapy of *H. pylori*-associated gastric disorders [117].

Statin, a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor

Statins are commonly used lipid-lowering drugs that reduce the risk of cardiovascular morbidity and mortality. Although recent studies have pointed to chemopreventive effects of statins against various cancers, their efficacy for gastric cancer is unclear. Thus, pitavastatin, a lipophilic statin, was examined on *H. pylori*-associated stomach carcinogenesis and gastritis using Mongolian gerbil and mouse models. The incidences of *H. pylori*-associated gastric adenocarcinomas and degrees of chronic gastritis were not decreased by pitavastatin compared with those of control values. Expression of IL-1β and TNFα mRNAs in the pyloric mucosa was markedly upregulated in pitavastatin-treated animals. Furthermore, in the *H. pylori*-infected groups, serum total cholesterol, triglyceride, and low-density lipoprotein levels were significantly increased by pitavastatin treatment, contrary to expectation. In the short-term study, *H. pylori*-infected gerbils and mice also showed significant upregulation of serum triglyceride levels by pitavastatin, whereas total cholesterol was markedly reduced and low-density lipoprotein exhibited a tendency for decrease in non-infected animals. These findings indicate pitavastatin to be ineffective for suppressing gastritis and chemoprevention of gastric carcinogenesis in *H. pylori*-infected gerbils. Our serologic results also suggest that the *H. pylori* infection and consequent severe chronic gastritis interfere with the cholesterol-lowering effects of pitavastatin [116].

Intestinal metaplasia and intestinalization of gastric cancer

Human gastric-and-intestinal-mixed-type intestinal metaplasia: aberrant expression of transcription factors

H. pylori plays a causative role in the development of chronic atrophic gastritis and intestinal metaplasia as well as stomach neoplasms. Although intestinal metaplasia has long attracted attention as a putative preneoplastic lesion for stomach cancers, its clinicopathologic significance has yet to be clarified in detail. Using gastric and intestinal epithelial cell markers, intestinal metaplasia was here divided into two major types: a gastric-and-intestinal-mixed type and a solely intestinal type [31, 121]. In the former, gastric and intestinal phenotypic markers appeared not only within the glandular but also at the same cellular level [66]. Furthermore, neuroendocrine cells also showed intestinalization along with their exocrine counterparts [74]. The molecular mechanisms of intestinal metaplasia include the ectopic expression of CDX1/CDX2 [2, 56, 88], OCT-1 [32], and members of the Erk pathway. Suppression of the expression of gastric transcription factors such as SOX2 [121], genes that are involved in the Sonic hedgehog pathway, and RUNX3 [71], a tumor suppressor gene, could be additional relevant alterations. The expression of PDX1 may also be associated with pseudopyloric gland metaplasia and intestinal metaplasia [84]. Detailed analysis of gene regulation may shed light on the molecular bases of gastric lesions, leading to strategies for chemoprevention [122].

Intestinal metaplasia in experimental animals

Experimentally, a phenotypic shift from gastric-and-intestinal-mixed-type intestinal metaplasia to solely intestinal type could be clearly observed on sequential observation of rat stomach treated with X-rays [138]. In Mongolian gerbil model, gastric-and-intestinal-mixed-type intestinal metaplasia was found to appear first, followed by the solely intestinal type with appearance of Paneth cells during the overall course of *H. pylori* infection in the HPGs [69].

Summarizing these data, it was suggested that intestinal metaplasia might be caused by the gradual intestinalization of stem cells from the gastric-and-intestinal-mixed type to the solely intestinal type.

Intestinalization of adenocarcinoma

Human gastric adenocarcinomas have been classified by Lauren into two major groups, the "intestinal" and "diffuse" types [45], which respectively nearly correspond to the "differentiated" and "undifferentiated" types [65, 91]. However, the above-mentioned classifications are inadequate for

studies of histogenesis of gastric carcinomas and phenotype expression at the cellular level because they confuse intestinal phenotypic cancer cells with a "diffuse" structure and gastric phenotypic ones with the "intestinal" type of Lauren [113]. The phenotypic expression of gastric cancer cells of each histological type can be clearly classified into gastric and intestinal epithelial cell types by immunohistochemistry using gastric and intestinal epithelial cell markers such as MUC5AC, MUC6, MUC2, and villin, independent of the histological type (Table 2) [16, 37, 40, 57–59, 98, 113, 135]. Gastric cancers comprising epithelial elements presenting only gastric or intestinal phenotypic expression are classified as of gastric, or intestinal phenotypes, respectively. Those with both gastric type cells and intestinal type cells have a gastric-and-intestinal-mixed phenotype, while the remainder exhibiting neither are grouped as a null type [37, 57, 98, 108, 135].

It has been suggested that "intestinal" type carcinomas arise in intestinalized mucosa, whereas the "diffuse" type develops from the gastric mucosa proper [11, 13, 45, 65] and a number of authors have proposed that intestinal metaplasia is a precancerous lesion for differentiated type gastric cancers [11, 12, 63, 89, 136, 137]. However, this hypothesis is based on morphological similarities between cancers and intestinal metaplasia in the surrounding mucosa and previous studies on phenotypic expression of each intestinal metaplastic or stomach cancer cells have pointed to several contradictions [16, 24, 37, 44, 57, 59, 83, 105–108]. In both experimental animals and humans, gastric cancers at early stages, independent of the histological type, mainly consist of gastric type cancer cells, and a phenotypic shift from gastric to intestinal phenotypic expression is clearly observed with progression [4, 104–106, 109, 113, 131]. When the phenotypic classification is compared in early and advance stomach cancers, shift from gastric toward intestinal, and then null phenotypes was observed [57, 59] (Fig. 4a). Regarding the histogenesis of gastric cancers, it would be logical if those originating from intestinal metaplasia should be of the intestinal type. Even if the phenotypic expression of intestinal type gastric cancer cells is unstable, the incidence of intestinal type cancer cells in small gastric cancers should then be higher than in large gastric cancers, the opposite from the actual case, and expression in fact appears to be stable [106]. In addition, on analysis of microsatellite instability, Tamura et al. [102] found that the majority of differentiated adenocarcinomas of the stomach may develop through a de novo pathway from the viewpoint of the microsatellite alterations. Endoh et al. [17] also clarified that genetic backgrounds of differentiated type tumors were quite different among cellular phenotypes. Thus, it has been proposed that intestinal metaplasia is important not as a precancerous lesion but as a paracancerous phenomenon [16, 54, 108]. Therefore, many questions remain regarding its pathogenesis as well as the actual relationship to gastric cancers.

Gastric and intestinal phenotypic expression in stomach cancers in carcinogen-treated and *H. pylori*-infected Mongolian gerbils

The *H. pylori*-infected *Mongolian gerbil* has been established as an appropriate animal model for studies of stomach cancer development. However, there have hitherto been no data on the phenotypic classification of glandular stomach cancers in *H. pylori*-infected and non-infected Mongolian gerbils. Thus, the phenotypes of 50 and six advanced glandular stomach cancers in *H. pylori*-infected and non-infected gerbils, respectively, were analyzed using several gastrointestinal epithelial phenotypic markers. The lesions were divided phenotypically into 21 gastric, 24 gastric-and-intestinal mixed, four intestinal and one null types, with 90.0 % of the lesions harboring gastric elements and

Table 2 The phenotypic markers for gastrointestinal epithelial cells

Tissue types	Cell types	Markers for human tissues	Markers for Mongolian gerbils
Gastric	Foveolar	MUC5AC	Human gastric mucin (HGM)
		Periodic acid-Schiff staining (PAS)	PAS
	Pyloric	MUC6	PCS
		Paradoxical concanavalin A staining (PCS)	
Intestinal	Goblet	MUC2	Small intestinal mucinous antigen (SIMA)
		Alcian blue	Alcian blue
		CDX2	
	Absorptive	Villin	Intestinal type alkaline phoshate (I-ALP)
		CD 10	CD 10
		CDX2	

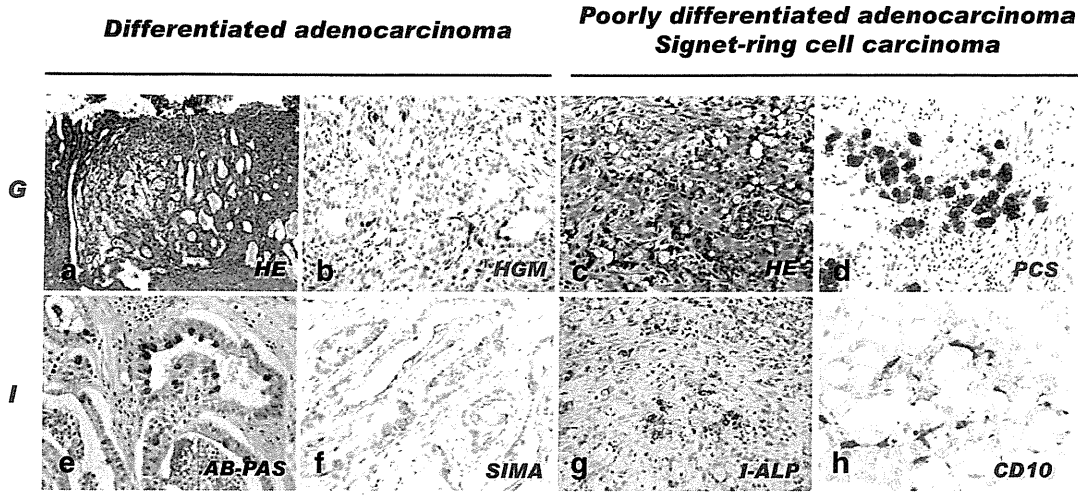

Fig. 3 Adenocarcinomas with gastric or intestinal phenotypic expression in MNU-induced *H. pylori*-infected Mongolian gerbils. **a**, **b**, **e**, **f** Differentiated adenocarcinoma. **c**, **d**, **g**, **h** Poorly differentiated adenocarcinoma/signet-ring cell carcinoma. **a**, **b**, **c**, **d** Gastric type (*G, colored red*). **e**, **f**, **g**, **h** Intestinal type (*I, colored blue*). **a**, **c** HE staining. **d** Paradoxical concanavalin A staining (*PCS*). **e** Alcian blue-periodic acid-Schiff (*AB-PAS*) staining. **b**, **f**, **g**, **h** Immunohistochemistry with antibodies against human gastric mucin (*HGM*) (**b**), small intestinal mucinous antigen (*SIMA*) (**f**), and intestinal-alkaline phosphatase (*I-ALP*) (**g**), and CD10 (**h**) (Table 2). Reproduced from Ref. [60]with permission

56.0 % demonstrating intestinal phenotypic expression in *H. pylori*-infected Mongolian gerbils. All six lesions were classified as gastric type in non-infected gerbils. There was no clear correlation with the presence of intestinal metaplasia in surrounding mucosa. Most of the advanced adenocarcinomas retain a gastric cellular phenotype, suggesting intestinal metaplasia as a paracancerous phenomenon rather than a premalignant condition. On the other hand, more than half of the cancers harbor intestinal phenotypes only in *H. pylori*-infected group compared with none in uninfected cancers with statistical significance. *H. pylori* infection was considered to trigger intestinalization of both stomach cancers and non-neoplastic mucosa (Figures 3 and 4b, Table 2) [60].

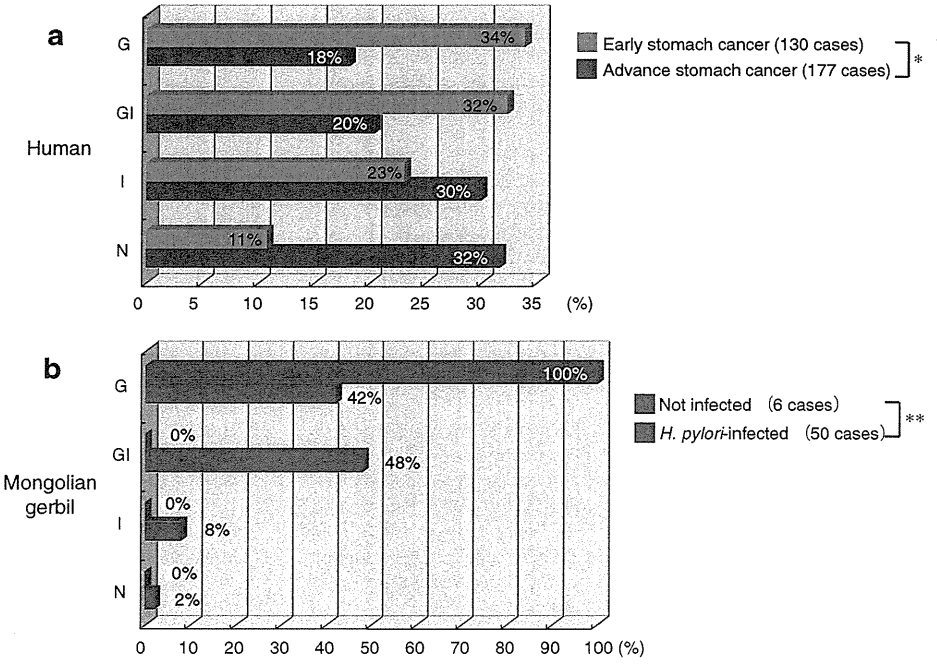

Fig. 4 Intestinalization of stomach cancers with *H. pylori* infection: **a** Phenotypic classification of human stomach cancers in early [59] (*orange bars*) and advanced cases [57] (*Burgundy bars*). Transition is apparent from gastric (*G*), gastric-and-intestinal mixed (*GI*), intestinal (*I*), and toward null (*N*) phenotypes in advanced cancers compared to early cases. $*P<0.0001$, χ^2 test for trend. **b** Phenotypic classification of stomach cancers in Mongolian gerbils. *H. pylori* infection (*red bars*) induced intestinalization of stomach cancers, whereas non-infected animals developed those with only gastric phenotype (*blue bars*)[59]. $**P<0.02$, χ^2 test for trend,

Conclusions

H. pylori infection is a very important factor for gastric carcinogenesis in human stomach. Since the discovery of *H. pylori*, the Mongolian gerbil has become one of the most important model animal for analysis of stomach carcinogenesis and trials of chemoprevention. As revealed by the experimental models described above, it was clarified that *H. pylori* itself only causes chronic inflammation and acts as promoter in stomach carcinogenesis. Further analyses need be conducted to determine mechanisms of carcinogenesis and contribute to chemopreventive methods.

References

1. Adlercreutz H (2002) Phyto-oestrogens and cancer. Lancet Oncol 3:364–373
2. Almeida R, Silva E, Santos-Silva F, Silberg DG, Wang J, De Bolos C, David L (2003) Expression of intestine-specific transcription factors, CDX1 and CDX2, in intestinal metaplasia and gastric carcinomas. J Pathol 199:36–40
3. Asaka M, Kato M, Kudo M, Katagiri M, Nishikawa K, Koshiyama H, Takeda H, Yoshida J, Graham DY (1996) Atrophic changes of gastric mucosa are caused by *Helicobacter pylori* infection rather than aging: studies in asymptomatic Japanese adults. Helicobacter 1:52–56
4. Bamba M, Sugihara H, Kushima R, Okada K, Tsukashita S, Horinouchi M, Hattori T (2001) Time-dependent expression of intestinal phenotype in signet ring cell carcinomas of the human stomach. Virchows Arch 438:49–56
5. Cao X, Tsukamoto T, Nozaki K, Tanaka H, Shimizu N, Kaminishi M, Kumagai T, Tatematsu M (2002) Earlier *Helicobacter pylori* infection increases the risk for the *N*-methyl-*N*-nitrosourea-induced stomach carcinogenesis in Mongolian gerbils. Jpn J Cancer Res 93:1293–1298
6. Cao X, Tsukamoto T, Nozaki K, Shimizu N, Mizoshita T, Kumagai T, Kaminishi M, Tatematsu M (2004) Eradication of *Helicobacter pylori* induces apoptosis and inhibits proliferation of heterotopic proliferative glands in infected Mongolian gerbils. Cancer Sci 95:872–877
7. Cao X, Tsukamoto T, Nozaki K, Tanaka H, Cao L, Toyoda T, Takasu S, Ban H, Kumagai T, Tatematsu M (2007) Severity of gastritis determines glandular stomach carcinogenesis in *Helicobacter pylori*-infected Mongolian gerbils. Cancer Sci 98:478–483
8. Cao X, Tsukamoto T, Seki T, Tanaka H, Morimura S, Cao L, Mizoshita T, Ban H, Toyoda T, Maeda H, Tatematsu M (2008) 4-Vinyl-2,6-dimethoxyphenol (canolol) suppresses oxidative stress and gastric carcinogenesis in *Helicobacter pylori*-infected carcinogen-treated Mongolian gerbils. Int J Cancer 122:1445–1454
9. Cao L, Mizoshita T, Tsukamoto T, Takenaka Y, Toyoda T, Cao X, Ban H, Nozaki K, Tatematsu M (2008) Development of carcinoid tumors of the glandular stomach and effects of eradication in *Helicobacter pylori*-infected mongolian gerbils. Asian Pac J Cancer Prev 9:25–30
10. Chiu CH, McEntee MF, Whelan J (1997) Sulindac causes rapid regression of preexisting tumors in Min/+ mice independent of prostaglandin biosynthesis. Cancer Res 57:4267–4273
11. Correa P (1988) A human model of gastric carcinogenesis. Cancer Res 48:3554–3560
12. Correa P (1992) Human gastric carcinogenesis: a multistep and multifactorial process–First American Cancer Society Award Lecture on Cancer Epidemiology and Prevention. Cancer Res 52:6735–6740
13. Correa P (1995) *Helicobacter pylori* and gastric carcinogenesis. Am J Surg Pathol 19(Suppl 1):S37–S43
14. Correa P, Fontham ET, Bravo JC, Bravo LE, Ruiz B, Zarama G, Realpe JL, Malcom GT, Li D, Johnson WD, Mera R (2000) Chemoprevention of gastric dysplasia: randomized trial of antioxidant supplements and anti-*Helicobacter pylori* therapy. J Natl Cancer Inst 92:1881–1888
15. Craanen ME, Dekker W, Blok P, Ferwerda J, Tytgat GN (1992) Intestinal metaplasia and *Helicobacter pylori*: an endoscopic bioptic study of the gastric antrum. Gut 33:16–20
16. Egashira Y, Shimoda T, Ikegami M (1999) Mucin histochemical analysis of minute gastric differentiated adenocarcinoma. Pathol Int 49:55–61
17. Endoh Y, Sakata K, Tamura G, Ohmura K, Ajioka Y, Watanabe H, Motoyama T (2000) Cellular phenotypes of differentiated-type adenocarcinomas and precancerous lesions of the stomach are dependent on the genetic pathways. J Pathol 191:257–263
18. Forman D, Newell DG, Fullerton F, Yarnell JW, Stacey AR, Wald N, Sitas F (1991) Association between infection with *Helicobacter pylori* and risk of gastric cancer: evidence from a prospective investigation. BMJ 302:1302–1305
19. Fox JG, Li X, Cahill RJ, Andrutis K, Rustgi AK, Odze R, Wang TC (1996) Hypertrophic gastropathy in Helicobacter felis-infected wild-type C57BL/6 mice and p53 hemizygous transgenic mice. Gastroenterology 110:155–166
20. Fukase K, Kato M, Kikuchi S, Inoue K, Uemura N, Okamoto S, Terao S, Amagai K, Hayashi S, Asaka M (2008) Effect of eradication of *Helicobacter pylori* on incidence of metachronous gastric carcinoma after endoscopic resection of early gastric cancer: an open-label, randomised controlled trial. Lancet 372:392–397
21. Furihata C, Ohta H, Katsuyama T (1996) Cause and effect between concentration-dependent tissue damage and temporary cell proliferation in rat stomach mucosa by NaCl, a stomach tumor promoter. Carcinogenesis 17:401–406
22. Futagami S, Suzuki K, Hiratsuka T, Shindo T, Hamamoto T, Tatsuguchi A, Ueki N, Shinji Y, Kusunoki M, Wada K, Miyake K, Gudis K, Tsukui T, Sakamoto C (2006) Celecoxib inhibits Cdx2 expression and prevents gastric cancer in *Helicobacter pylori*-infected Mongolian gerbils. Digestion 74:187–198
23. Graham DY, Lew GM, Klein PD, Evans DG, Evans DJ Jr, Saeed ZA, Malaty HM (1992) Effect of treatment of *Helicobacter pylori* infection on the long-term recurrence of gastric or duodenal ulcer. A randomized, controlled study. Ann Intern Med 116:705–708
24. Hattori T (1986) Development of adenocarcinomas in the stomach. Cancer 57:1528–1534
25. Hirayama F, Takagi S, Yokoyama Y, Iwao E, Ikeda Y (1996) Establishment of gastric *Helicobacter pylori* infection in Mongolian gerbils. J Gastroenterol 31(Suppl 9):24–28
26. Hirayama F, Takagi S, Iwao E, Yokoyama Y, Haga K, Hanada S (1999) Development of poorly differentiated adenocarcinoma and carcinoid due to long-term *Helicobacter pylori* colonization in Mongolian gerbils. J Gastroenterol 34:450–454
27. Honda S, Fujioka T, Tokieda M, Satoh R, Nishizono A, Nasu M (1998) Development of *Helicobacter pylori*-induced gastric carcinoma in Mongolian gerbils. Cancer Res 58:4255–4259
28. Hu PJ, Li YY, Zhou MH, Chen MH, Du GG, Huang BJ, Mitchell HM, Hazell SL (1995) *Helicobacter pylori* associated with a high prevalence of duodenal ulcer disease and a low prevalence of gastric cancer in a developing nation. Gut 36:198–202
29. Huang JQ, Sridhar S, Chen Y, Hunt RH (1998) Meta-analysis of the relationship between *Helicobacter pylori* seropositivity and gastric cancer. Gastroenterology 114:1169–1179

30. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans (1994) Infection with *Helicobacter pylori*. Schistosomes, liver flukes and *Helicobacter pylori*. World Health Organization/International Agency for Research on Cancer, Lyon, pp 177–241
31. Inada K, Nakanishi H, Fujimitsu Y, Shimizu N, Ichinose M, Miki K, Nakamura S, Tatematsu M (1997) Gastric and intestinal mixed and solely intestinal types of intestinal metaplasia in the human stomach. Pathol Int 47:831–841
32. Jin T, Li H (2001) Pou homeodomain protein OCT1 is implicated in the expression of the caudal-related homeobox gene Cdx-2. J Biol Chem 276:14752–14758
33. Joossens JV, Hill MJ, Elliott P, Stamler R, Lesaffre E, Dyer A, Nichols R, Kesteloot H (1996) Dietary salt, nitrate and stomach cancer mortality in 24 countries. European Cancer Prevention (ECP) and the INTERSALT Cooperative Research Group. Int J Epidemiol 25:494–504
34. Karita M, Kouchiyama T, Okita K, Nakazawa T (1991) New small animal model for human gastric *Helicobacter pylori* infection: success in both nude and euthymic mice. Am J Gastroenterol 86:1596–1603
35. Karita M, Li Q, Cantero D, Okita K (1994) Establishment of a small animal model for human *Helicobacter pylori* infection using germ-free mouse. Am J Gastroenterol 89:208–213
36. Kato S, Tsukamoto T, Mizoshita T, Tanaka H, Kumagai T, Ota H, Katsuyama T, Asaka M, Tatematsu M (2006) High salt diets dose-dependently promote gastric chemical carcinogenesis in *Helicobacter pylori*-infected Mongolian gerbils associated with a shift in mucin production from glandular to surface mucous cells. Int J Cancer 119:1558–1566
37. Kawachi H, Takizawa T, Eishi Y, Shimizu S, Kumagai J, Funata N, Koike M (2003) Absence of either gastric or intestinal phenotype in microscopic differentiated gastric carcinomas. J Pathol 199:436–446
38. Kawakubo M, Ito Y, Okimura Y, Kobayashi M, Sakura K, Kasama S, Fukuda MN, Fukuda M, Katsuyama T, Nakayama J (2004) Natural antibiotic function of a human gastric mucin against *Helicobacter pylori* infection. Science 305:1003–1006
39. Kono S, Hirohata T (1996) Nutrition and stomach cancer. Cancer Causes Control 7:41–55
40. Koseki K, Takizawa T, Koike M, Ito M, Nihei Z, Sugihara K (2000) Distinction of differentiated type early gastric carcinoma with gastric type mucin expression. Cancer 89:724–732
41. Krakowka S, Morgan DR, Kraft WG, Leunk RD (1987) Establishment of gastric *Campylobacter pylori* infection in the neonatal gnotobiotic piglet. Infect Immun 55:2789–2796
42. Kuipers EJ, Uyterlinde AM, Pena AS, Roosendaal R, Pals G, Nelis GF, Festen HP, Meuwissen SG (1995) Long-term sequelae of *Helicobacter pylori* gastritis. Lancet 345:1525–1528
43. Kurihara M, Shirakabe H, Murakami T, Yasui A, Izumi T (1974) A new method for producing adenocarcinomas in the stomach of dogs with *N*-ethyl-*N'*-nitro-*N*-nitrosoguanidine. Gann 65:163–177
44. Kushima R, Hattori T (1993) Histogenesis and characteristics of gastric-type adenocarcinomas in the stomach. J Cancer Res Clin Oncol 120:103–111
45. Lauren P (1965) The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma: an attempt at a histo-clinical classification. Acta Pathol Microbiol Scand 64:31–49
46. Lee A, Fox JG, Otto G, Murphy J (1990) A small animal model of human *Helicobacter pylori* active chronic gastritis. Gastroenterology 99:1315–1323
47. Lee A, O'Rourke J, De Ungria MC, Robertson B, Daskalopoulos G, Dixon MF (1997) A standardized mouse model of *Helicobacter pylori* infection: introducing the Sydney strain. Gastroenterology 112:1386–1397
48. Leung WK, Sung JJ (2006) Chemoprevention of gastric cancer. Eur J Gastroenterol Hepatol 18:867–871
49. Magari H, Shimizu Y, Inada K, Enomoto S, Tomeki T, Yanaoka K, Tamai H, Arii K, Nakata H, Oka M, Utsunomiya H, Tsutsumi Y, Tsukamoto T, Tatematsu M, Ichinose M (2005) Inhibitory effect of etodolac, a selective cyclooxygenase-2 inhibitor, on stomach carcinogenesis in *Helicobacter pylori*-infected Mongolian gerbils. Biochem Biophys Res Commun 334:606–612
50. Malaty HM, El-Kasabany A, Graham DY, Miller CC, Reddy SG, Srinivasan SR, Yamaoka Y, Berenson GS (2002) Age at acquisition of *Helicobacter pylori* infection: a follow-up study from infancy to adulthood. Lancet 359:931–935
51. Marchetti M, Arico B, Burroni D, Figura N, Rappuoli R, Ghiara P (1995) Development of a mouse model of *Helicobacter pylori* infection that mimics human disease. Science 267:1655–1658
52. Marshall BJ, Warren JR (1984) Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. Lancet:1311–1315
53. Matsubara S, Takasu S, Tsukamoto T, Mutoh M, Masuda S, Sugimura T, Wakabayashi K, Totsuka Y (2012) Induction of glandular stomach cancers in *Helicobacter pylori*-infected Mongolian gerbils by 1-nitrosoindole-3-acetonitrile. Int J Cancer 130:259–266
54. Matsukuma A, Mori M, Enjoji M (1990) Sulphomucin-secreting intestinal metaplasia in the human gastric mucosa. An association with intestinal-type gastric carcinoma. Cancer 66:689–694
55. Miyazawa M, Utsunomiya H, Inada K, Yamada T, Okuno Y, Tanaka H, Tatematsu M (2006) Inhibition of *Helicobacter pylori* motility by (+)-Syringaresinol from unripe Japanese apricot. Biol Pharm Bull 29:172–173
56. Mizoshita T, Inada K, Tsukamoto T, Kodera Y, Yamamura Y, Hirai T, Kato T, Joh T, Itoh M, Tatematsu M (2001) Expression of Cdx1 and Cdx2 mRNAs and relevance of this expression to differentiation in human gastrointestinal mucosa–with special emphasis on participation in intestinal metaplasia of the human stomach. Gastric Cancer 4:185–191
57. Mizoshita T, Tsukamoto T, Nakanishi H, Inada K, Ogasawara N, Joh T, Itoh M, Yamamura Y, Tatematsu M (2003) Expression of Cdx2 and the phenotype of advanced gastric cancers: relationship with prognosis. J Cancer Res Clin Oncol 129:727–734
58. Mizoshita T, Inada K, Tsukamoto T, Nozaki K, Joh T, Itoh M, Yamamura Y, Ushijima T, Nakamura S, Tatematsu M (2004) Expression of the intestine-specific transcription factors, Cdx1 and Cdx2, correlates shift to an intestinal phenotype in gastric cancer cells. J Cancer Res Clin Oncol 130:29–36
59. Mizoshita T, Tsukamoto T, Inada K, Ogasawara N, Hirata A, Kato S, Joh T, Itoh M, Yamamura Y, Tatematsu M (2004) Immunohistochemically detectable Cdx2 is present in intestinal phenotypic elements in early gastric cancers of both differentiated and undifferentiated types, with no correlation to non-neoplastic surrounding mucosa. Pathol Int 54:392–400
60. Mizoshita T, Tsukamoto T, Takenaka Y, Cao X, Kato S, Kaminishi M, Tatematsu M (2006) Gastric and intestinal phenotypes and histogenesis of advanced glandular stomach cancers in carcinogen-treated, *Helicobacter pylori*-infected Mongolian gerbils. Cancer Sci 97:38–44
61. Mori K (1967) Carcinoma of the glandular stomach of mice by instillation of 4-nitroquinoline 1-oxide. Gann 58:389–393
62. Mori K, Ohta A (1967) Carcinoma of the glandular stomach of mice induced by 4-hydroxyaminoquinoline 1-oxide. Gann 58:551–554
63. Morson BC (1955) Carcinoma arising from areas of intestinal metaplasia in the gastric mucosa. Br J Cancer 9:377–385
64. Naito Y, Yoshikawa T (2002) Molecular and cellular mechanisms involved in *Helicobacter pylori*-induced inflammation and oxidative stress. Free Radic Biol Med 33:323–336

65. Nakamura K, Sugano H, Takagi K (1968) Carcinoma of the stomach in incipient phase: its histogenesis and histological appearances. Gann 59:251–258
66. Niwa T, Ikehara Y, Nakanishi H, Tanaka H, Inada K, Tsukamoto T, Ichinose M, Tatematsu M (2005) Mixed gastric- and intestinal-type metaplasia Is formed by cells with dual intestinal and gastric differentiation. J Histochem Cytochem 53:75–85
67. Nomura A, Stemmermann GN, Chyou PH, Kato I, Perez-Perez GI, Blaser MJ (1991) *Helicobacter pylori* infection and gastric carcinoma among Japanese Americans in Hawaii. N Engl J Med 325:1132–1136
68. Nozaki K, Shimizu N, Inada K, Tsukamoto T, Inoue M, Kumagai T, Sugiyama A, Mizoshita T, Kaminishi M, Tatematsu M (2002) Synergistic promoting effects of *Helicobacter pylori* infection and high-salt diet on gastric carcinogenesis in Mongolian gerbils. Jpn J Cancer Res 93:1083–1089
69. Nozaki K, Shimizu N, Tsukamoto T, Inada K, Cao X, Ikehara Y, Kaminishi M, Sugiyama A, Tatematsu M (2002) Reversibility of heterotopic proliferative glands in glandular stomach of *Helicobacter pylori*-infected Mongolian gerbils on eradication. Jpn J Cancer Res 93:374–381
70. Nozaki K, Shimizu N, Ikehara Y, Inoue M, Tsukamoto T, Inada K, Tanaka H, Kumagai T, Kaminishi M, Tatematsu M (2003) Effect of early eradication on *Helicobacter pylori*-related gastric carcinogenesis in Mongolian gerbils. Cancer Sci 94:235–239
71. Osaki M, Moriyama M, Adachi K, Nakada C, Takeda A, Inoue Y, Adachi H, Sato K, Oshimura M, Ito H (2004) Expression of RUNX3 protein in human gastric mucosa, intestinal metaplasia and carcinoma. Eur J Clin Investig 34:605–612
72. Oshima H, Oshima M, Inaba K, Taketo MM (2004) Hyperplastic gastric tumors induced by activated macrophages in COX-2/mPGES-1 transgenic mice. EMBO J 23:1669–1678
73. Oshima H, Matsunaga A, Fujimura T, Tsukamoto T, Taketo MM, Oshima M (2006) Carcinogenesis in mouse stomach by simultaneous activation of the Wnt signaling and prostaglandin E2 pathway. Gastroenterology 131:1086–1095
74. Otsuka T, Tsukamoto T, Mizoshita T, Inada K, Takenaka Y, Kato S, Yamamura Y, Miki K, Tatematsu M (2005) Coexistence of gastric- and intestinal-type endocrine cells in gastric and intestinal mixed intestinal metaplasia of the human stomach. Pathol Int 55:170–179
75. Otsuka T, Tsukamoto T, Tanaka H, Inada K, Utsunomiya H, Mizoshita T, Kumagai T, Katsuyama T, Miki K, Tatematsu M (2005) Suppressive effects of fruit-juice concentrate of *Prunus mume* Sieb. et Zucc. (Japanese apricot, Ume) on *Helicobacter pylori*-induced glandular stomach lesions in Mongolian gerbils. Asian Pac J Cancer Prev 6:337–341
76. Parkin DM, Bray F, Ferlay J, Pisani P (2005) Global cancer statistics, 2002. CA Cancer J Clin 55:74–108
77. Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, Sibley RK (1991) *Helicobacter pylori* infection and the risk of gastric carcinoma. N Engl J Med 325:1127–1131
78. Parsonnet J, Hansen S, Rodriguez L, Gelb AB, Warnke RA, Jellum E, Orentreich N, Vogelman JH, Friedman GD (1994) *Helicobacter pylori* infection and gastric lymphoma. N Engl J Med 330:1267–1271
79. Prescott SM, Fitzpatrick FA (2000) Cyclooxygenase-2 and carcinogenesis. Biochim Biophys Acta 1470:M69–M78
80. Radin MJ, Eaton KA, Krakowka S, Morgan DR, Lee A, Otto G, Fox J (1990) *Helicobacter pylori* gastric infection in gnotobiotic beagle dogs. Infect Immun 58:2606–2612
81. Reddy BS, Maruyama H, Kelloff G (1987) Dose-related inhibition of colon carcinogenesis by dietary piroxicam, a nonsteroidal antiinflammatory drug, during different stages of rat colon tumor development. Cancer Res 47:5340–5346
82. Rusch HP, Baumann CA, Maison GL (1940) Production of internal tumors with chemical carcinogens. Arch Pathol 29:8–19
83. Saito A, Shimoda T, Nakanishi Y, Ochiai A, Toda G (2001) Histologic heterogeneity and mucin phenotypic expression in early gastric cancer. Pathol Int 51:165–171
84. Sakai H, Eishi Y, Li XL, Akiyama Y, Miyake S, Takizawa T, Konishi N, Tatematsu M, Koike M, Yuasa Y (2004) PDX1 homeobox protein expression in pseudopyloric glands and gastric carcinomas. Gut 53:323–330
85. Shimizu N, Inada K, Nakanishi H, Tsukamoto T, Ikehara Y, Kaminishi M, Kuramoto S, Sugiyama A, Katsuyama T, Tatematsu M (1999) *Helicobacter pylori* infection enhances glandular stomach carcinogenesis in Mongolian gerbils treated with chemical carcinogens. Carcinogenesis 20:669–676
86. Shimizu N, Inada KI, Tsukamoto T, Nakanishi H, Ikehara Y, Yoshikawa A, Kaminishi M, Kuramoto S, Tatematsu M (1999) New animal model of glandular stomach carcinogenesis in Mongolian gerbils infected with *Helicobacter pylori* and treated with a chemical carcinogen. J Gastroenterol 34(Suppl 11):61–66
87. Shimizu N, Ikehara Y, Inada K, Nakanishi H, Tsukamoto T, Nozaki K, Kaminishi M, Kuramoto S, Sugiyama A, Katsuyama T, Tatematsu M (2000) Eradication diminishes enhancing effects of *Helicobacter pylori* infection on glandular stomach carcinogenesis in Mongolian gerbils. Cancer Res 60:1512–1514
88. Silberg DG, Furth EE, Taylor JK, Schuck T, Chiou T, Traber PG (1997) CDX1 protein expression in normal, metaplastic, and neoplastic human alimentary tract epithelium. Gastroenterology 113:478–486
89. Stemmermann GN, Hayashi T (1968) Intestinal metaplasia of the gastric mucosa: a gross and microscopic study of its distribution in various disease states. J Natl Cancer Inst 41:627–634
90. Stewart HL, Snell KC (1958) Histopathogenesis of carcinoma induced in the glandular stomach of C57BL mice by the intramural injection of 20-methylcholanthrene. J Natl Cancer Inst 21:999–1035
91. Sugano H, Nakamura K, Kato Y (1982) Pathological studies of human gastric cancer. Acta Pathol Jpn 32(Suppl 2):329–347
92. Sugimura T, Fujimura S (1967) Tumour production in glandular stomach of rat by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. Nature 216:943–944
93. Sugimura T, Kawachi T (1973) In: Busch H (ed) Methods in cancer research, 7th edn. Academic Press Inc, New York, pp 245–308
94. Sugimura T, Kawachi T (1976) Experimental gastric cancer (author's transl). Leber Magen Darm 6:80–90
95. Sugiyama A, Maruta F, Ikeno T, Ishida K, Kawasaki S, Katsuyama T, Shimizu N, Tatematsu M (1998) *Helicobacter pylori* infection enhances *N*-methyl-*N*-nitrosourea-induced stomach carcinogenesis in the Mongolian gerbil. Cancer Res 58:2067–2069
96. Takahashi M (1970) Effect of alkylbenzenesulfonate as a vehicle for 4-nitroquinoline 1-oxide on gastric carcinogenesis in rats. Gann 61:27–33
97. Tajima K, Tominaga S (1985) Dietary habits and gastro-intestinal cancers: a comparative case–control study of stomach and large intestinal cancers in Nagoya, Japan. Jpn J Cancer Res 76:705–716
98. Tajima Y, Shimoda T, Nakanishi Y, Yokoyama N, Tanaka T, Shimizu K, Saito T, Kawamura M, Kusano M, Kumagai K (2001) Gastric and intestinal phenotypic marker expression in gastric carcinomas and its prognostic significance: immunohistochemical analysis of 136 lesions. Oncology 61:212–220
99. Takahashi M, Sato H (1969) Effect of 4-nitroquinoline 1-oxide with alkylbenzenesulfonate on gastric carcinogenesis in rats. Gann Monogr 8:241–261
100. Takahashi M, Nishikawa A, Furukawa F, Enami T, Hasegawa T, Hayashi Y (1994) Dose-dependent promoting effects of sodium chloride (NaCl) on rat glandular stomach carcinogenesis initiated with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. Carcinogenesis 15:1429–1432

101. Takasu S, Tsukamoto T, Cao XY, Toyoda T, Hirata A, Ban H, Yamamoto M, Sakai H, Yanai T, Masegi T, Oshima M, Tatematsu M (2008) Roles of cyclooxygenase-2 and microsomal prostaglandin E synthase-1 expression and beta-catenin activation in gastric carcinogenesis in *N*-methyl-*N*-nitrosourea-treated K19-mE transgenic mice. Cancer Sci 99:2356–2364
102. Tamura G, Sakata K, Maesawa C, Suzuki Y, Terashima M, Satoh K, Sekiyama S, Suzuki A, Eda Y, Satodate R (1995) Microsatellite alterations in adenoma and differentiated adenocarcinoma of the stomach. Cancer Res 55:1933–1936
103. Tatematsu M, Takahashi M, Fukushima S, Hananouchi M, Shirai T (1975) Effects in rats of sodium chloride on experimental gastric cancers induced by *N*-methyl-*N*-nitro-*N*-nitrosoguanidine or 4-nitroquinoline-1-oxide. J Natl Cancer Inst 55:101–106
104. Tatematsu M, Katsuyama T, Fukushima S, Takahashi M, Shirai T, Ito N, Nasu T (1980) Mucin histochemistry by paradoxical concanavalin A staining in experimental gastric cancers induced in Wistar rats by *N*-methyl-*N*-nitro-*N*-nitrosoguanidine or 4-nitroquinoline 1-oxide. J Natl Cancer Inst 64:835–843
105. Tatematsu M, Furihata C, Katsuyama T, Hasegawa R, Nakanowatari J, Saito D, Takahashi M, Matsushima T, Ito N (1983) Independent induction of intestinal metaplasia and gastric cancer in rats treated with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. Cancer Res 43:1335–1341
106. Tatematsu M, Katsuyama T, Furihata C, Tsuda H, Ito N (1984) Stable intestinal phenotypic expression of gastric and small intestinal tumor cells induced by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine or methylnitrosourea in rats. Gann 75:957–965
107. Tatematsu M, Furihata C, Katsuyama T, Miki K, Honda H, Konishi Y, Ito N (1986) Gastric and intestinal phenotypic expressions of human signet ring cell carcinomas revealed by their biochemistry, mucin histochemistry, and ultrastructure. Cancer Res 46:4866–4872
108. Tatematsu M, Ichinose M, Miki K, Hasegawa R, Kato T, Ito N (1990) Gastric and intestinal phenotypic expression of human stomach cancers as revealed by pepsinogen immunohistochemistry and mucin histochemistry. Acta Pathol Jpn 40:494–504
109. Tatematsu M, Katsuyama T, Furihata C, Fukushima S, Shirai T, Kato T, Ito N (1990) Cellular differentiation and histogenesis of rat glandular stomach cancers. Jpn J Cancer Res 81:760–767
110. Tatematsu M, Ogawa K, Hoshiya T, Shichino Y, Kato T, Imaida K, Ito N (1992) Induction of adenocarcinomas in the glandular stomach of BALB/c mice treated with *N*-methyl-*N*-nitrosourea. Jpn J Cancer Res 83:915–918
111. Tatematsu M, Yamamoto M, Iwata H, Fukami H, Yuasa H, Tezuka N, Masui T, Nakanishi H (1993) Induction of glandular stomach cancers in C3H mice treated with *N*-methyl-*N*-nitrosourea in the drinking water. Jpn J Cancer Res 84:1258–1264
112. Tatematsu M, Yamamoto M, Shimizu N, Yoshikawa A, Fukami H, Kaminishi M, Oohara T, Sugiyama A, Ikeno T (1998) 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 89:97–104
113. Tatematsu M, Tsukamoto T, Inada K (2003) Stem cells and gastric cancer—role of gastric and intestinal mixed intestinal metaplasia. Cancer Sci 94:135–141
114. The EUROGAST Study Group (1993) An international association between *Helicobacter pylori* infection and gastric cancer. Lancet 341:1359–1362
115. Toyoda T, Tsukamoto T, Mizoshita T, Nishibe S, Deyama T, Takenaka Y, Hirano N, Tanaka H, Takasu S, Ban H, Kumagai T, Inada K, Utsunomiya H, Tatematsu M (2007) Inhibitory effect of nordihydroguaiaretic acid, a plant lignan, on *Helicobacter pylori*-associated gastric carcinogenesis in Mongolian gerbils. Cancer Sci 98:1689–1695
116. Toyoda T, Tsukamoto T, Takasu S, Hirano N, Ban H, Shi L, Kumagai T, Tanaka T, Tatematsu M (2009) Pitavastatin fails to lower serum lipid levels or inhibit gastric carcinogenesis in *Helicobacter pylori*-infected rodent models. Cancer Prev Res (Phila, PA) 2:751–758
117. Toyoda T, Tsukamoto T, Takasu S, Shi L, Hirano N, Ban H, Kumagai T, Tatematsu M (2009) Anti-inflammatory effects of caffeic acid phenethyl ester (CAPE), a nuclear factor-kappaB inhibitor, on *Helicobacter pylori*-induced gastritis in Mongolian gerbils. Int J Cancer 125:1786–1795
118. Tsugane S, Tsuda M, Gey F, Watanabe S (1992) Cross-sectional study with multiple measurements of biological markers for assessing stomach cancer risks at the population level. Environ Health Perspect 98:207–210
119. Tsugane S (2005) Salt, salted food intake, and risk of gastric cancer: epidemiologic evidence. Cancer Sci 96:1–6
120. Tsugane S, Sasazuki S (2007) Diet and the risk of gastric cancer: review of epidemiological evidence. Gastric Cancer 10:75–83
121. Tsukamoto T, Inada K, Tanaka H, Mizoshita T, Mihara M, Ushijima T, Yamamura Y, Nakamura S, Tatematsu M (2004) Down regulation of a gastric transcription factor, Sox2, and ectopic expression of intestinal homeobox genes, Cdx1 and Cdx2: inverse correlation during progression from gastric/intestinal-mixed to complete intestinal metaplasia. J Cancer Res Clin Oncol 130:135–145
122. Tsukamoto T, Mizoshita T, Tatematsu M (2006) Gastric-and-intestinal mixed-type intestinal metaplasia: aberrant expression of transcription factors and stem cell intestinalization. Gastric Cancer 9:156–166
123. Tsukamoto T, Mizoshita T, Tatematsu M (2007) Animal models of stomach carcinogenesis. Toxicol Pathol 35:636–648
124. Tsukamoto H, Mizoshita T, Sasaki M, Mizushima T, Tanida S, Ozeki K, Hirata Y, Shimura T, Kataoka H, Kamiya T, Nojiri S, Tsukamoto T, Tatematsu M, Joh T (2011) Long-term high-dose proton pump inhibitor administration to *Helicobacter pylori*-infected Mongolian gerbils enhances neuroendocrine tumor development in the glandular stomach. Asian Pac J Cancer Prev 12:1049–1054
125. Uemura N, Mukai T, Okamoto S, Yamaguchi S, Mashiba H, Taniyama K, Sasaki N, Haruma K, Sumii K, Kajiyama G (1997) Effect of *Helicobacter pylori* eradication on subsequent development of cancer after endoscopic resection of early gastric cancer. Cancer Epidemiol Biomarkers Prev 6:639–642
126. Uemura N, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M, Taniyama K, Sasaki N, Schlemper RJ (2001) *Helicobacter pylori* infection and the development of gastric cancer. N Engl J Med 345:784–789
127. Warren JR, Marshall B (1983) Unidentified curved bacilli on gastric epithelium in active chronic gastritis. Lancet 321:1273–1275
128. Watanabe T, Tada M, Nagai H, Sasaki S, Nakao M (1998) *Helicobacter pylori* infection induces gastric cancer in mongolian gerbils. Gastroenterology 115:642–648
129. Wilson RH, De Eds F, Cox AJ Jr (1941) The toxicity and carcinogenic activity of 2-acetaminofluorene. Cancer Res 1:595–608
130. Wong BC, Lam SK, Wong WM, Chen JS, Zheng TT, Feng RE, Lai KC, Hu WH, Yuen ST, Leung SY, Fong DY, Ho J, Ching CK (2004) *Helicobacter pylori* eradication to prevent gastric cancer in a high-risk region of China: a randomized controlled trial. Jama 291:187–194
131. Yamachika T, Inada K, Fujimitsu Y, Nakamura S, Yamamura Y, Kitou T, Itzkowitz SH, Werther JL, Miki K, Tatematsu M (1997) Intestinalization of gastric signet ring cell carcinomas with progression. Virchows Arch 431:103–110

132. Yamamoto M, Tsukamoto T, Sakai H, Shirai N, Ohgaki H, Furihata C, Donehower LA, Yoshida K, Tatematsu M (2000) p53 knockout mice (−/−) are more susceptible than (+/−) or (+/+) mice to *N*-methyl-*N*-nitrosourea stomach carcinogenesis. Carcinogenesis 21:1891–1897
133. Yamamoto M, Furihata C, Ogiu T, Tsukamoto T, Inada K, Hirano K, Tatematsu M (2002) Independent variation in susceptibilities of six different mouse strains to induction of pepsinogen-altered pyloric glands and gastric tumor intestinalization by *N*-methyl-*N*-nitrosourea. Cancer Lett 179:121–132
134. Yanaoka K, Oka M, Yoshimura N, Deguchi H, Mukoubayashi C, Enomoto S, Maekita T, Inoue I, Ueda K, Utsunomiya H, Iguchi M, Tamai H, Fujishiro M, Nakamura Y, Tsukamoto T, Inada K, Takeshita T, Ichinose M (2010) Preventive effects of etodolac, a selective cyclooxygenase-2 inhibitor, on cancer development in extensive metaplastic gastritis, a *Helicobacter pylori*-negative precancerous lesion. Int J Cancer 126:1467–1473
135. Yoshikawa A, Inada Ki K, Yamachika T, Shimizu N, Kaminishi M, Tatematsu M (1998) Phenotypic shift in human differentiated gastric cancers from gastric to intestinal epithelial cell type during disease progression. Gastric Cancer 1:134–141
136. You WC, Blot WJ, Li JY, Chang YS, Jin ML, Kneller R, Zhang L, Han ZX, Zeng XR, Liu WD et al (1993) Precancerous gastric lesions in a population at high risk of stomach cancer. Cancer Res 53:1317–1321
137. Yuasa Y (2003) Control of gut differentiation and intestinal-type gastric carcinogenesis. Nat Rev Cancer 3:592–600
138. Yuasa H, Inada K, Watanabe H, Tatematsu M (2002) A phenotypic shift from gastric-intestinal to solely intestinal cell types in intestinal metaplasia in rat stomach following treatment with X-rays. J Toxicol Pathol 15:85–93