

## P53 Overexpression in Gastric Adenocarcinoma with *Helicobacter pylori* Infection

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*Gastric carcinogenesis has been studied in various aspects. Helicobacter pylori (Hp) infection and mutation of the p53 tumor suppressor gene have recently been argued to be important factors of gastric carcinogenesis. There have been many studies to determine the precise mechanism of how Hp is related to gastric cancer, but it is so far still unknown. We studied the relationship of Hp infection and p53 overexpression and tried to discover some significance in clinico-pathologic factors such as age, sex, stage, site, differentiation and gross morphology. Ninety-six patients who were diagnosed with gastric cancer at Severance Hospital, Yonsei University Medical College from November 1995 to March 1996, and 96 control patients of non-ulcer dyspepsia (NUD) were studied by endoscopic biopsy of normal gastric tissue and cancer tissue. They also underwent the CLO<sup>®</sup> (Delta West, Melbourne, Western Australia) test for Hp positivity and p53 immunohistochemical stain for p53 positivity. These data were analyzed for comparison with the clinicopathologic characteristics of gastric cancers. In conclusion, the differentiated group cancer had a significantly high Hp positivity and p53 positivity. There is a possibility that Hp infection and p53 tumor suppressor gene mutation might be significantly related in the gastric carcinogenic process of well- and moderately-differentiated adenocarcinomas, but further study is necessary to determine more direct clues on the carcinogenic roles of these factors.*

**Key Words :** Gastric cancer, *Helicobacter pylori*, p53 overexpression

Despite the high mortality of gastric cancer, the etiology of the disease is still uncertain. Food, environment, and genetic aspects have been the fields

of active investigation (Lipkin, 1992). Until 1983, the main determinant of the gastric microenvironment responsible for oncogenic cell transformations was believed to be related to food (Correa, 1983). After the first report that *Helicobacter pylori* (Hp) may be related to gastric carcinogenesis (Marshall and Warren, 1984), Hp was studied as another important risk factor for gastric cancer. Hp was then reported to be related to gastric cancer in 3 prospective case-control studies (Forman *et al.* 1991; Nomura *et al.* 1991; Parsonnet *et al.* 1991), and a study of 17 patient populations in 13 European countries showed that an Hp-infected patient had a 6-times higher risk of gastric cancer compared to a non-infected patient (Forman *et al.* 1993). Another

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study reported that the duration of Hp infection and the risk of gastric cancer was proportionally related (Forman *et al.* 1994). Finally, the International Agency for Research on Cancer confirmed Hp as an infectious group 1 carcinogen along with *Hepatitis B virus*, *Human papilloma virus*, *Schistosoma haematobium*, and *Opisthorchis viverrini* (International Agency for Research on Cancer, 1994).

There have been many attempts to determine how Hp is involved in gastric carcinogenesis. Hp-infected normal tissue aggregates polymorphonuclear cells and progresses to chronic gastritis where the gastric mucosa is atrophied and develops atrophic gastritis, which in turn transforms to intestinal metaplasia and through dysplasia changes to gastric cancer tissue. This is the chronological model proposed by Correa in 1995 (Correa, 1995). In atrophic gastritis, gastric acid and pepsin secretion is decreased, which increases the gastric pH and the growth of the bacteria. Nitrates are converted to nitrites by the reductase of the bacteria which eventually stimulates mitosis of the gastric mucosal cells and turns to premalignant lesions such as intestinal metaplasia or dysplasia (Keefer and Roller, 1973). Hp also converts urea to ammonia by *H. pylori* urease and stimulates mitosis (Tsuji *et al.* 1992). Vacuolating cytotoxin, acetaldehyde, cytokines and various biological mediators secreted by Hp stimulate growth and differentiation of epithelial cells, and accelerate gastric carcinogenesis (O'Connor, 1992).

Among the many inflammatory cells infiltrated in the gastric mucosal tissue, polymorphonuclear cells and monocytes are the major cells that migrate through the epithelial cells from the interstitium to the gastric lumen. A proportion of the polymorphonuclear cells are degenerated and "oxidative bursts" release nitric oxides and hydroxyl radicals, which eventually mutate the DNA of the dividing epithelial cells (Nguyen *et al.* 1992). This mutation may occur on various genes such as the p53 tumor suppressor gene. The polymorphonuclear cells are also infiltrated near the cells which have already gone through dysplasia and may cause additional mutations (Correa, 1992). The wild type of p53 gene suppresses cell growth and transformations, but its small quantity and short half-life makes it difficult to be viewed by immunohistochemical stain (Rogel *et al.* 1985). The mutant type of p53 gene under-

goes a structural change and its long half-life enables a large amount of intracellular accumulation which makes it possible to be revealed by immunohistochemical stain (Bartek *et al.* 1990; Gannon *et al.* 1990; Rodrigues *et al.* 1990).

We have investigated the overexpression of the p53 tumor suppressor gene by immunohistochemical stain of the mutant type of p53 gene. By examining the Hp infectivity, we tried to find a certain relationship between Hp infection and the p53 tumor suppressor gene mutation, and to make a comparison with various clinicopathologic characteristics of the gastric cancers.

## MATERIALS AND METHODS

### Materials

The subjects were 96 patients who were diagnosed with gastric adenocarcinoma at Yonsei University Severance Hospital from November 1995 to March 1996 and 96 control patients who were randomly chosen from non-ulcer dyspepsia (NUD) patients by esophagogastroduodenoscopy during the same period of time. Among the gastric cancer patients, the male-to-female ratio was 1.6:1, and the average age was 56.1 years. There were 54 early gastric cancer patients and 42 advanced gastric cancer patients. The control patients had no history of gastric surgery, no history of chronic drug use, no history of systemic diseases, and no abnormalities found on routine physical examination.

### Methods

Hp positivity was measured in gastric cancer patients by endoscopic biopsy and CLO<sup>®</sup> (Delta West, Melbourne, Western Australia) test of the normal gastric mucosa and the mucosa near the cancer tissue. In the control patients, 2 mucosal biopsies were taken from the fundus and the pylorus and the CLO<sup>®</sup> test was performed.

P53 immunohistochemical staining was done by the following method. The pathology slides were re-examined and selected paraffin blocks were cut in 4  $\mu$ m thickness. Mouse monoclonal antibody clone DO7 (Novocastra laboratories, Newcastle, UK) was

used for antibodies of the p53 stain. The paraffin blocks were deparaffinized by xylene and rehydrated by sequentially concentrated alcohol, and endogenous peroxidase activity was blocked by 0.3% hydrogen peroxide. To decrease nonspecific binding, the tissue was combined with normal goat serum for 20 minutes and bound with primary antibody diluted by 1:100 for 1 hour at room temperature. After PBS buffer washing, the tissue was reacted with biotinylated antibody for 30 minutes at room temperature, washed again with PBS buffer for 10 minutes and reacted with Novostain Super ABC Reagent for 30 minutes at room temperature. The DAB substrate peroxidase was spread for 2-7 minutes and the slides were stained by hematoxylin-eosin for 1 minute. After washing in tap water the slides were finally dried and mounted. P53 overexpression was read positive in cells with a stained nucleus and was read negative in cells with an unstained nucleus.

#### Statistics

SPSS was used for  $\chi^2$  test continuity correction and multivariate logistic regression analysis. Statistical significance was defined by the p-value of less than 0.05.

## RESULTS

Among the 96 gastric cancer patients, 65 patients underwent surgery, 31 patients underwent endoscopic biopsy only, 9 patients refused surgery, 15 patients were treated by chemotherapy, 5 patients were conservatively treated, and 2 patients died. The male-to-female ratio of the cancer patients was 59 to 37 (1.6:1). The age ranged from 28-to-77 years with an average age of 56.1 years. There was no significant statistical difference in the sex ratio and age distribution in the 96 control patients.

Hp positivity in the gastric cancer patients was 75 cases (78.1%) and in the control patients, 55 cases (57.3%). Hp positivity was significantly high in the gastric cancer patients compared to the control patients ( $p < 0.05$ ). P53 overexpression in gastric cancer patients was 46.9% and in control patients, 5.2%. P53 positivity was significantly high in gastric cancer patients compared to control patients ( $p < 0.01$ ). The positively-stained nucleus of p53 immunohistochemical stain in gastric adenocarcinoma is shown in Fig. 1. Among the gastric cancer patients, 42 cases (43.8%) were EGC (early gastric cancer) and

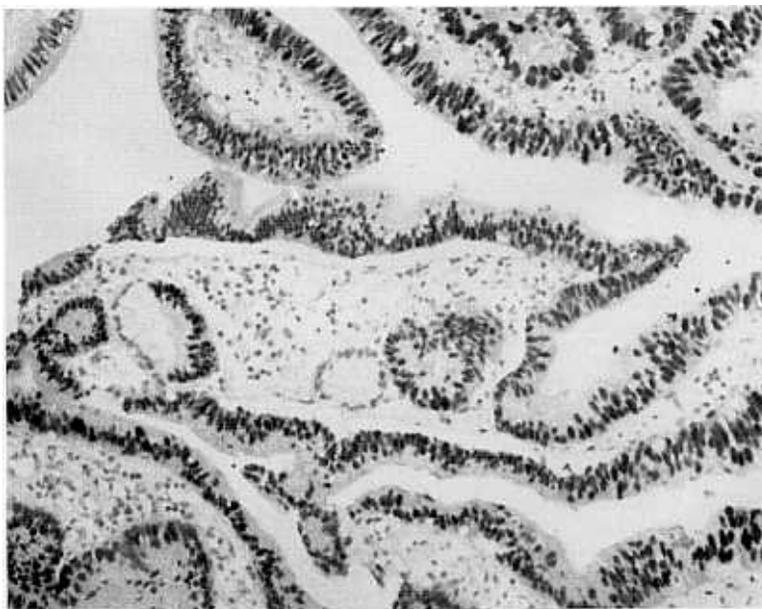
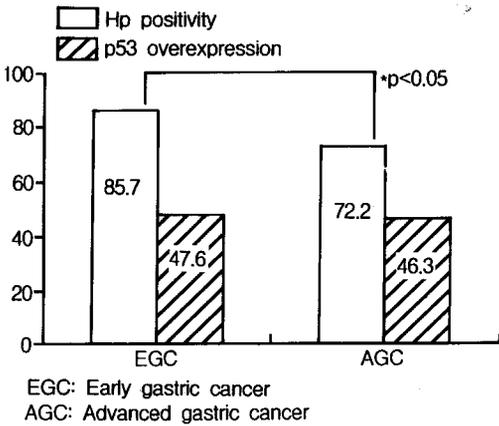


Fig. 1. Positively-stained nucleus of p53 immunohistochemical stain in gastric adenocarcinoma ( $\times 100$ ).



EGC: Early gastric cancer  
AGC: Advanced gastric cancer  
Fig. 2. *Helicobacter pylori* positivity and p53 overexpression in EGC and AGC.

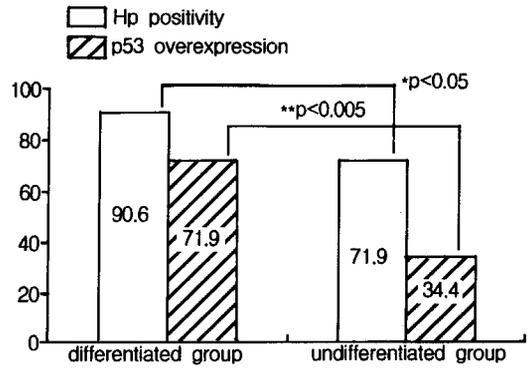


Fig. 3. *Helicobacter pylori* positivity and p53 overexpression in differentiated and undifferentiated group adenocarcinoma.

Table 1. Hp and p53 positivity in EGC and AGC

	EGC	AGC	Total
Hp positivity	36/42 (85.7%)*	39/54 (72.2%)	75/96 (78.1%)
p53 positivity	20/42 (47.6%)	25/54 (46.3%)	45/96 (46.9%)

Hp : *Helicobacter pylori*  
EGC: early gastric cancer  
AGC: advanced gastric cancer  
\* : p<0.05

54 cases (56.3%) were AGC (advanced gastric cancer) patients. Hp positivity was 85.7% (36 cases) and 72.2% (39 cases) respectively and Hp positivity was significantly high in the EGC patients (p<0.05). P53 positivity was 46.3% in AGC and 47.6% in EGC and there was no significant relationship with Hp positivity (Fig. 2)(Table 1). The gastric cancer patients were divided into 2 age groups. Thirty cases were below the age 40, and 66 cases were over 40. Hp positivity was 93.3% (28 cases) in the former group and 71.2% (47 cases) in the latter group. Hp positivity was significantly lower in the higher age group (p<0.05), but they were not related to p53 positivity. The sex ratio of the gastric cancer patients was 59 men and 37 women, and Hp positivity was 78.0% (46 cases) and 78.4% (29 cases) respectively, with no significant difference between the 2 groups. There was no significant difference in p53 positivity between men and women. Among the gastric cancer patients, moderately-differentiated adenocarcinoma

was the most frequent type of cellular differentiation. Well-differentiated and moderately-differentiated adenocarcinoma, which makes up the differentiated group, were 32 cases (33.2%), and poorly-differentiated adenocarcinoma, signet ring cell carcinoma and mucinous adenocarcinoma, which makes up the undifferentiated group, were 64 cases (66.7%). Hp positivity in the differentiated group and undifferentiated group was 90.6% (29 cases) and 71.9% (46 cases) respectively, and Hp positivity in the differentiated group was significantly high compared with the undifferentiated group (p<0.05). P53 positivity in the differentiated group was 71.9% (23 cases) and was significantly high (p<0.005) compared with the undifferentiated group, which was 34.4% (22 cases). Hp positivity and p53 positivity were both significantly high (p<0.05) in the differentiated group cancer (Fig. 3)(Table 2). The most frequent site of gastric cancer was 55 cases (57.3%) in the lower 1/3. Hp positivity in the lower 1/3 was

**Table 2. Hp and p53 positivity according to gross and microscopic morphology, location, and stage**

Parameter	Class	Hp (+)	(%)	p-value	p53 (+)	(%)	p-value	
Cellular differentiation	Differentiated	29/32	(90.6)	p<0.05	23/32	(71.9)	p<0.005	
	Undifferentiated	46/64	(71.9)		22/64	(34.4)		
Location	Upper 1/3	3/5	(60.0)	NS	1/5	(20.0)	NS	
	Middle 1/3	24/32	(75.0)		17/32	(53.1)		
	Lower 1/3	45/55	(81.8)		26/55	(47.3)		
	Diffuse	3/4	(75.0)		1/4	(25.0)		
TNM stage	1	16/20	(80.0)	NS	11/20	(55.0)	NS	
	2	18/24	(75.0)		12/24	(50.0)		
	3	21/26	(80.8)		11/26	(42.3)		
	4	20/26	(76.9)		11/26	(42.3)		
Gross morphology	EGC	protruded	11/13	NS	6/13	(46.2)	NS	
		nonprotruded	25/29		(86.2)	14/29		(48.3)
	AGC	protruded	6/8		(75.0)	4/8		(50.0)
		nonprotruded	33/46		(71.7)	21/46		(45.7)

Differentiated group: well-differentiated, moderately-differentiated, papillary adenocarcinoma

Undifferentiated group: poorly-differentiated, signet ring cell ca., mucinous adenocarcinoma

EGC : early gastric cancer --- protruded : I, IIa  
 --- nonprotruded : IIb, IIc, III

AGC : advanced gastric cancer --- protruded : Borrmann 1  
 --- nonprotruded : Borrmann 2,3,4

Hp : *Helicobacter pylori*

NS : not significant

45 cases (81.8%), middle 1/3 was 24 cases out of 32 cases (75.0%), upper 1/3 was 3 cases out of 5 cases (60.0%), and diffuse gastric cancer was 3 out of 4 cases (75.0%). There was no significant difference in Hp positivity and p53 positivity according to site (Table 2). Stages of gastric cancer were 20 cases (20.8%) in stage 1, 24 cases (25.0%) in stage 2, and 26 cases (27.1%) in stages 3 and 4. Hp positivity was 80.0% (16 cases) in stage 1, 75.0% (18 cases) in stage 2, 80.8% (21 cases) in stage 3, and 76.9% (20 cases) in stage 4, which showed no relationship between Hp positivity and stage. There was no significant relationship between p53 positivity and stage (Table 2). According to gross morphology, EGC type I and IIa were classified as protruded type, and IIb, IIc, and III were classified as nonprotruded type. Hp positivity in the protruded type was 11 cases (84.6%) and in the nonprotruded type it was 25 cases (86.2%). In AGC, Borrmann type 1 was classified as protruded type and Borrmann type 2, 3, and 4 were classified as nonprot-

ruded type. Hp positivity in the protruded type was 6 cases (75.0%) and in the nonprotruded type was 33 cases (71.7%) with no statistical significance. P53 positivity was not related to gross morphology (Table 2).

In the Hp positive group of gastric adenocarcinoma, p53 overexpression was not significantly positive in any of the variables of age, sex, site, stage or gross morphology. Among the Hp positive group, p53 overexpression was significantly high in the well- and moderately-differentiated adenocarcinomas, which make up the differentiated group of cellular differentiation (p<0.05)(Table 3). To analyze the simultaneous positivity of *Helicobacter* and p53 overexpression, logistic regression was run and the p values were statistically significant as 0.0244 in univariate analysis and 0.0470 in multivariate analysis in the differentiated group cancers of cellular differentiation.

**Table 3. Relationship between *Helicobacter pylori* positivity and p53 overexpression according to gross and microscopic morphology**

Parameter	Class	Hp positive		Hp negative	
		p53 (+)	p53 (-)	p53 (+)	p53 (-)
Gross morphology	EGC protruded	5 (45.5)	6 (54.5)	1 (50.0)	1 (50.0)
	EGC nonprotruded	12 (48.0)	13 (52.0)	2 (50.0)	2 (50.0)
	AGC protruded	3 (50.0)	3 (50.0)	1 (50.0)	1 (50.0)
	AGC nonprotruded	18 (54.5)	15 (45.5)	3 (23.1)	10 (76.9)
Cellular differentiation	Differentiated*	21 (72.4)	8 (27.6)	2 (66.7)	1 (33.3)
	Undifferentiated	17 (36.9)	29 (63.1)	5 (27.8)	13 (72.2)

\* : p&lt;0.05

Hp : *Helicobacter pylori*

Differentiated group : well-differentiated, moderately-differentiated, papillary adenocarcinoma

Undifferentiated group : poorly-differentiated, signet ring cell ca., mucinous adenocarcinoma

EGC : early gastric cancer --- protruded : I, IIa

--- nonprotruded : IIb, IIc, III

AGC : advanced gastric cancer --- protruded : Borrmann 1

--- nonprotruded : Borrmann 2,3,4

## DISCUSSION

There have been studies of the relationship between Hp positive gastric cancer and various gene mutations such as the TGF- $\beta$  RII, p16 and p53 gene. Mismatch repair failure has been confirmed to cause mutation on the poly(A) tract of TGF- $\beta$  RII gene and is significantly related to the intestinal-type gastric carcinoma in Hp positive patients (Chung *et al.* 1996). We specifically chose the p53 tumor suppressor gene among the many genes that may undergo mutations in gastric carcinogenesis to discover any relationship between Hp infection and p53 gene mutation by comparing various clinicopathologic characteristics such as the age, sex, stage, site, gross and microscopic morphology of the gastric cancers. Our study revealed a higher p53 overexpression and Hp positivity in well- and moderately-differentiated adenocarcinomas. Such a relationship may suggest the possibility that Hp infection triggers p53 gene mutation in a part of the gastric carcinogenic process. The question as to which stage p53 gene mutation may be involved and how Hp is related should be studied by investigating Hp positivity and p53 overexpression in each premalignant lesion, such as

atrophic gastritis, intestinal metaplasia and dysplasia. The degenerated features of the polymorphonuclear cells, the appearance and amount of free radicals and the destroyed features of the DNA should be confirmed in each of the premalignant stages in order to learn the detailed carcinogenic mechanism of gastric cancer. There was a study to actually determine the mechanistic link between Hp infection and gastric cancer. Increased oxidative DNA damage in Hp-infected gastric cancer was demonstrated by measuring the 8-hydroxydeoxyguanosine content in the DNA of human gastric mucosa, which is a marker for oxygen-free-radical-induced DNA damage (Baik *et al.* 1996). Some studies show a direct proportion of the dysplastic degree and the amount of p53 overexpression, where in low-grade dysplasias there is no p53 overexpression and in high-grade dysplasias there is abundant p53 overexpression (Rugge *et al.* 1992; Lauwers *et al.* 1993; Tahara *et al.* 1993). These results may permit speculation that p53 mutation has a role in the end stage of dysplasia.

In our study, according to cellular differentiation, the differentiated group, which includes the well-differentiated and moderately-differentiated adenocarcinomas, has a significantly higher Hp positivity and

p53 overexpression compared to the undifferentiated group which includes the poorly-differentiated adenocarcinoma and signet ring cell carcinoma. The relationship of cellular differentiation and the p53 gene has been thoroughly debated. A study of 149 cases of gastric cancer showed p53 overexpression in 4% of signet ring cell carcinoma and 43% of non-signet ring cell carcinoma (Uchino *et al.* 1992). Another study of 118 cases had a p53 overexpression of 37% in well- and moderately-differentiated adenocarcinoma and 0% in poorly-differentiated adenocarcinoma and signet ring cell carcinoma (Noguchi *et al.* 1993). But there have also been reports of irrelevance between cellular differentiation and p53 mutation (Tamura *et al.* 1991; Starzynsky *et al.* 1992). A study discriminated two separate carcinogenic pathways which end up to be intestinal and diffuse gastric cancers. P53 gene mutation was restricted to the intestinal gastric cancer in the Hp-infected 205 early gastric cancers (Solcia *et al.* 1996). Our study had similar results showing increased p53 and Hp positivity in the well-differentiated and moderately-differentiated adenocarcinomas, which are mostly the intestinal-type gastric cancers.

Hp positivity increases with increasing age in the general population. On the other hand, Hp positivity decreases with increasing age in gastric cancer patients (Taylor and Blaser, 1991). Our study had the same result of low Hp positivity in higher age groups. The most appropriate explanation for this trend is the spontaneous disappearance of *Helicobacter pylori* microbials (Banatvala *et al.* 1993). Hp-infected gastric mucosa becomes atrophic gastritis with hypochlorhydria and transforms to intestinal metaplasia, which is a microenvironment unfavorable to the viability of Hp (Siurala *et al.* 1988). As time passes, *Helicobacter* will diminish from the gastric mucosa and will eventually show low Hp positivity in gastric cancer patients. Another possible explanation is the disappearance of Hp by the direct effect of the tumor cell, where the tumor growth forces out Hp from the tissue. For this mechanism to be credible, Hp positivity must decrease with a larger tumor mass and higher stage, but in our study, as well as in other studies, there have been no such relationships. There are reports of p53 overexpression increasing in higher age groups, but our study

showed no relationship between age and p53 gene mutation.

Whether early gastric cancer (EGC) progresses to advanced gastric cancer (AGC) is a controversial subject. Recent epidemiologic studies describe most untreated EGC as progressing to AGC in a period of 4-to-5 years (Tsukuma *et al.* 1983; Eckardt *et al.* 1990). In our study, while Hp positivity in EGC was significantly higher than the control, AGC was not so. This may be explained by the decrease of Hp as EGC progresses to AGC because of the unfavorable microenvironment made as the tumor grows larger (Karnes *et al.* 1991; Craanen *et al.* 1992). P53 overexpression had no specific relationship with either EGC or AGC in our study.

In conclusion, the differentiated group cancer had a significantly high Hp positivity and p53 positivity. There is a possibility that Hp infection and p53 tumor suppressor gene mutation might be significantly related in the gastric carcinogenic process of well- and moderately-differentiated adenocarcinomas, but further study is necessary for more direct clues on the carcinogenic roles of these factors.

## REFERENCES

- Baik SC, Youn HS, Chung MH, Lee WK, Cho MJ, Ko GH, Park CK, Kasai H, Rhee KH: Increased oxidative DNA damage in *Helicobacter pylori*-infected human gastric mucosa. *Cancer Res* 56: 1279-1282, 1996
- Bartek J, Iggo R, Gannon J, Lane DP: Genetic and immunochemical analysis of mutant p53 in human breast cancer cell lines. *Oncogene* 5: 893-899, 1990
- Banatvala N, Mayo K, Megraud F, Jennings R, Deeks JJ, Feldman RA: The cohort effect and *Helicobacter pylori*. *J Infect Dis* 168: 219-221, 1993
- Chung YJ, Song JM, Lee JY, Jung YT, Seo EJ, Choi SW, Rhyu MG: Microsatellite instability-associated mutations associate preferentially with the intestinal type of primary gastric carcinomas in a high-risk population. *Cancer Res* 56: 4662-4665, 1996
- Correa P: The gastric precancerous process. *Cancer Surg* 2: 437-450, 1983
- Correa P: Human gastric carcinogenesis; a multistep and multifactorial process. *Cancer Res* 52: 6735-6740, 1992
- Correa P: *Helicobacter pylori* and gastric carcinogenesis. *Am J Surg Pathol* 19(suppl 1): 37-43, 1995
- Craanen ME, Dekker W, Blok P, Ferwerda J, Tytgat GNJ: Intestinal metaplasia and *Helicobacter pylori*: an endoscopic bioptic study of the gastric antrum.

- Gut* 33: 16-20, 1992
- Eckardt VF, Giessler W, Kanzler G, Remmele W, Bernhard G: Clinical and morphological characteristics of early gastric cancer. *Gastroenterology* 98: 708-714, 1990
- Forman D, Coleman M, Debacker G: An international association between *Helicobacter pylori* infection and gastric cancer. *Lancet* 341: 1359-1362, 1993
- Forman D, Newell DG, Fullerton F, Yarnell JW, Stacey AR, Wald N, Sitas F: Association between infection with *Helicobacter pylori* and risk of gastric cancer: evidence from a prospective investigation. *Br Med J* 302: 1302-1305, 1991
- Forman D, Webb P, Parsonnet J: *Helicobacter pylori* and gastric cancer. *Lancet* 343: 243-244, 1994
- Gannon JV, Greaves R, Iggo R, Lane DP: Activating mutations in p53 produce a common conformational effect. *EMBO J* 9: 1595-1599, 1990
- International Agency for Research on Cancer: The evaluation of carcinogenic risks to humans. *World Health Organ Monograph* 61: 53-66, 1994
- Karnes WE Jr., Samloff IM, Siurala M, Kekki M, Sipponen P, Kim SWR: Positive serum antibody and negative tissue staining for *Helicobacter pylori* in subjects with atrophic body gastritis. *Gastroenterology* 101: 167-174, 1991
- Keefer LK, Roller PP: N-nitrosation by nitrite ion in neutral and basal medium. *Science* 181: 1245-1247, 1973
- Lauwers GY, Wahl SJ, Melamed J, Rojas-Corona RR: P53 expression in precancerous gastric lesions: An immunohistochemical study of PAb 1801 monoclonal antibody on adenomatous and hyperplastic gastric polyps. *Am J Gastroenterol* 88: 1916-1920, 1993
- Lipkin M: Gastrointestinal cancer: pathogenesis, risk factors and the development of intermediate biomarkers for chemoprevention studies. *J Cell Biochem* 2(suppl 16): 1-13, 1992
- Marshall BJ, Warren JR: Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. *Lancet* 1: 1311-1315, 1984
- Nguyen T, Brunson D, Crespi J: DNA damage and mutations in human cells exposed to nitric oxide in vitro. *Proc Natl Acad Sci USA* 89: 3030-3039, 1992
- Noguchi M, Ochiai A, Saito T, Kobayashi M, Hirohashi S: P53 mutation in gastric cancer: a genetic model for carcinogenesis is common to gastric and colorectal cancer. *Int J Cancer* 54: 759-767, 1993
- Nomura A, Stemmermann GN, Chyou PH, Kato I, Perez GI, Blaser MJ: *Helicobacter pylori* infection and gastric carcinoma among Japanese Americans in Hawaii. *N Engl J Med* 325: 1132-1136, 1991
- O'Connor HJ: *Helicobacter pylori* and gastric cancer: a review and hypothesis. *Eur J Gastroenterol Hepatol* 4: 103-109, 1992
- Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N, Sibley RK: *Helicobacter pylori* infection and the risk of gastric carcinoma. *N Engl J Med* 325: 1127-1131, 1991
- Rodrigues NR, Rowan A, Smith MEF, Kerr IB, Bodmer WF, Gannon JV, Lane DP: P53 mutations in colorectal cancer. *Proc Natl Acad Sci USA* 87: 7555-7561, 1990
- Rogel A, Poploker M, Webb CG, Oren M: P53 cellular tumor antigen: analysis of mRNA levels in normal adult tissues, embryos, and tumors. *Mol Cell Biol* 5: 2851-2859, 1985
- Rugge M, Shiao YH, Correa P, Baffa R, DiMario F: Immunohistochemical evidence of p53 overexpression in gastric epithelial dysplasia. *Cancer Epidemiol Biomarkers Prev* 1: 551-554, 1992
- Siurala M, Sipponen P, Kekki M: *Campylobacter pylori* in a sample of Finnish population: relations to morphology and functions of the gastric mucosa. *Gut* 29: 909-915, 1988
- Solcia E, Fiocca R, Luinetti O, Villani L, Padovan L, Calistri D, Ranzani GN, Chiaravalli A, Capella C: Intestinal and diffuse gastric cancers arise in a different background of *Helicobacter pylori* gastritis through different gene involvement. *Am J Surg Pathol* 20(suppl 1): S8-22, 1996
- Starzynsky T, Bromley M, Ghosh A, Stern PL: Prognostic significance of p53 overexpression in gastric and colorectal carcinoma. *Br J Cancer* 66: 558-564, 1992
- Tahara E, Kuniyasu H, Nakayama H, Yokozaki H: Metastasis related genes and malignancy in human esophageal, gastric and colorectal cancers. *Gan To Kagaku Ryoho* 20: 326-332, 1993
- Tamura G, Kihara T, Nomura K: Detection of frequent p53 gene mutations in primary gastric cancer by cell sorting and polymerase chain reaction single-stranded conformation polymorphism analysis. *Cancer Res* 51: 3056-3060, 1991
- Taylor DN, Blaser MJ: Epidemiology of *Helicobacter pylori* infections. *Epidemiol Rev* 13: 42-59, 1991
- Tsuji M, Kawano S, Tsujii S, Naganok K, Ito T, Hayashi N, Fusamoto H, Kamada T, Tamura K: Ammonia, a possible promoter in *Helicobacter pylori*-related gastric carcinogenesis. *Cancer Lett* 65: 15-18, 1992
- Tsukuma H, Mishima T, Oshima A: Prospective study of early gastric cancer. *Int J Cancer* 31: 421-426, 1983
- Uchino S, Noguchi M, Hirota T, Itabashi M, Saito T, Kobayashi M, Hirohashi S: High incidence of nuclear accumulation of p53 protein in gastric cancer. *Jpn J Clin Oncol* 22: 225-231, 1992