

9th Seah Cheng Siang Memorial Lecture: Gastric Cancer-Where are we now?†

S K Lam,**MD, FRCP, FHKCP*

Abstract

Gastric cancer, the second most common cancer in the world, kills about one million people a year, almost half of whom are Chinese. Chinese, Japanese and Koreans as well as east Europeans top the list with over 40 per 100,000 population per year, with a wide margin over Americans, Indians and Zimbabweans in whom the rates are below 1 per 100,000. The excellent prognosis of early gastric cancer is well established, and survival of cancer involving beyond the submucosa remains poor and there is little new in management. However, recent years have witnessed a breakthrough in the understanding of causative factors and molecular genetic abnormalities in gastric cancer that should pave the way for prevention, early detection and prognostication.

Established carcinogens for gastric cancer now include *Helicobacter pylori* and *N-nitroso* compounds; other causative factors include salt and salted food intake, cigarette smoking, male sex, and familial genetic abnormalities. *H. pylori* infection increases cancer risk by about 5 in a 10-year period. Diet high in salt carries a relative risk of up to 6, and a highly significant correlation between 24 h urinary salt content and incidence of gastric cancer has been shown in 24 countries. The risk from smoking and male sex is under 2. Many *N-nitroso* compounds, which come from nitrites, which in turn come from nitrates in food following bacterial transformation in a hypochlorhydric environment, are established carcinogens in animals, but their risk for human gastric cancer is still debatable.

The intestinal type of gastric cancer, according to Correa's hypothesis, develops from chronic inflammation leading to intestinal metaplasia, dysplasia and cancer, and is more associated with *H. pylori* and early gastric cancer. The diffuse type of gastric cancer does not go through these precancerous conditions and moves straight from inflammation to cancer. Associated with inflammation are an increase in proliferation and apoptosis, and this fine balance between proliferation and apoptosis may be uncoupled by genetic mutations. It is believed that as a result of the accumulation of molecular genetic abnormalities, a cancer eventually develops and metastasizes. *p53* mutation, cyclin overexpression (especially in intestinal type), microsatellite instability, down regulation of *E-cadherin* (especially in diffuse type), and telomerase reactivation are some prominent examples. These molecular abnormalities have the potential for screening, early detection and prognostication.

Fruits and vegetables, green tea, α -tocopherol and other micronutrients such as selenium have been shown to reduce the risk for gastric cancer. In fact, it has been reported that diet consisting of vegetables and fruits, low in salt, together with the avoidance of cigarette smoking would prevent two-thirds to three-quarters of gastric cancer. Furthermore, eradication of *H. pylori*, and for that matter future vaccination, has the theoretical potential of preventing gastric cancer, and the potential use of COX2 inhibiting NSAID in inducing apoptosis may reverse precancerous conditions of the stomach. Both approaches are being intensely studied.

Ann Acad Med Singapore 1999; 28:881-9

Key words: Dietary factor, Epidemiology, *Helicobacter pylori*, Molecular genetics

Introduction

The last two decades have witnessed dramatic changes in the understanding of gastric cancer: a rapid decline in global incidence, its association with *Helicobacter pylori* gastritis, and the new information regarding its molecular biology. Nevertheless, gastric cancer remains the second commonest cancer in the world in terms of morbidity and mortality, and takes away close to one million lives every year. This paper reviews the recent changes in the epidemiology and disease pattern, the

aetiological role of *H. pylori* and dietary factors, and the development of molecular biology in gastric cancer.

Epidemiology

Until recently, gastric cancer was the most frequently diagnosed cancer in the world. In the mid-1980s, gastric cancer was displaced by lung cancer and became the second commonest malignancy. The gastric cancer incidence has declined over the recent few decades. The decline took place globally,¹⁻⁵ occurring rapidly in north-

* Chairman of Medicine and Chief of Gastroenterology & Hepatology
Department of Medicine
University of Hong Kong
Queen Mary Hospital, Hong Kong

Address for Reprints: Dr Lam Shiu Kum, University Department of Hong Kong, 410 Professional Block, Queen Mary Hospital, Pokfulam, Hong Kong.

† Presented at the 19th Annual Combined Scientific Meeting of the Chapter of Physicians, Academy of Medicine, Singapore from 24 to 25 July 1999

ern Europe, steadily in North America, and only recently in East Asia (China, Japan and Korea). The cause for this decline in the incidence of gastric cancer is still a mystery and it occurred before the discovery and chemoradication of *H. pylori*. It is unknown if there has been a corresponding decline in the incidence of *H. pylori* infection as a result of improved environmental hygiene. It can also be argued that the decline is related to the invention of the refrigerator, which has led to a drastic reduction of salting fish, a common practice in northern European countries as a means to preserve the fish through winter following its catch in summer. Salt is an established dietary factor for gastric cancer (*vide infra*).

Reported mortality rates of gastric cancer vary widely:⁶ with low incidence (per 100,000 for males) in Zimbabwe (2.6), Mexico (5.5) and United States (6.4); high incidence in Japan (50.0), Russia (41.4) and China (26.2); and intermediate incidence in Denmark (12.0), Singapore (15.0) and Netherlands (15.9) (Fig. 1). The cumulative mortality rate can range from 1.17 to 82.83 per thousand population in different countries.⁷ In general, the incidence in Central and South America is much higher than that in North America, that in eastern European countries higher than that in west Europe, and that in east Asian countries (China, Japan and Korea) much higher

than that in the rest of Asia (Fig. 1).

Within a country, there are also considerable differences in incidence. Several parts of China have a very high incidence rate, such as Changle, Fujian (80 per 100,000), while in some parts the incidence is quite low. In fact, a north-south decreasing gradient has been recently observed in China, with northern China having a three-fold prevalence over the south.⁸ This gradient is also observed in other parts of the Northern Hemisphere.⁹⁻¹¹ In the Southern Hemisphere, the mortality risk tends to be higher in the southern part;^{12,13} it appears that higher geographic latitudes are associated with a higher gastric cancer risk. Even in a geographically small country such as Japan, there is also considerable variation in the incidence and mortality rates.¹⁴ In Singapore, the Chinese have a higher incidence than the Indians and Malays (Fig. 2), indicating strong ethnic differences. Ethnic differences also exist in the United States, where the incidence (in male per 100,000) in Blacks (14.5) and Hispanics (12) is about twice that in Whites (7).

The incidence rate of gastric cancer increases with age. This is observed in both high and low risk areas. For example, in the USA, the average incidence rate from 1973 to 1987 increased from 7.1 per 100,000 men aged 45-49 years to 135.7 per 100,000 men aged 85 years or

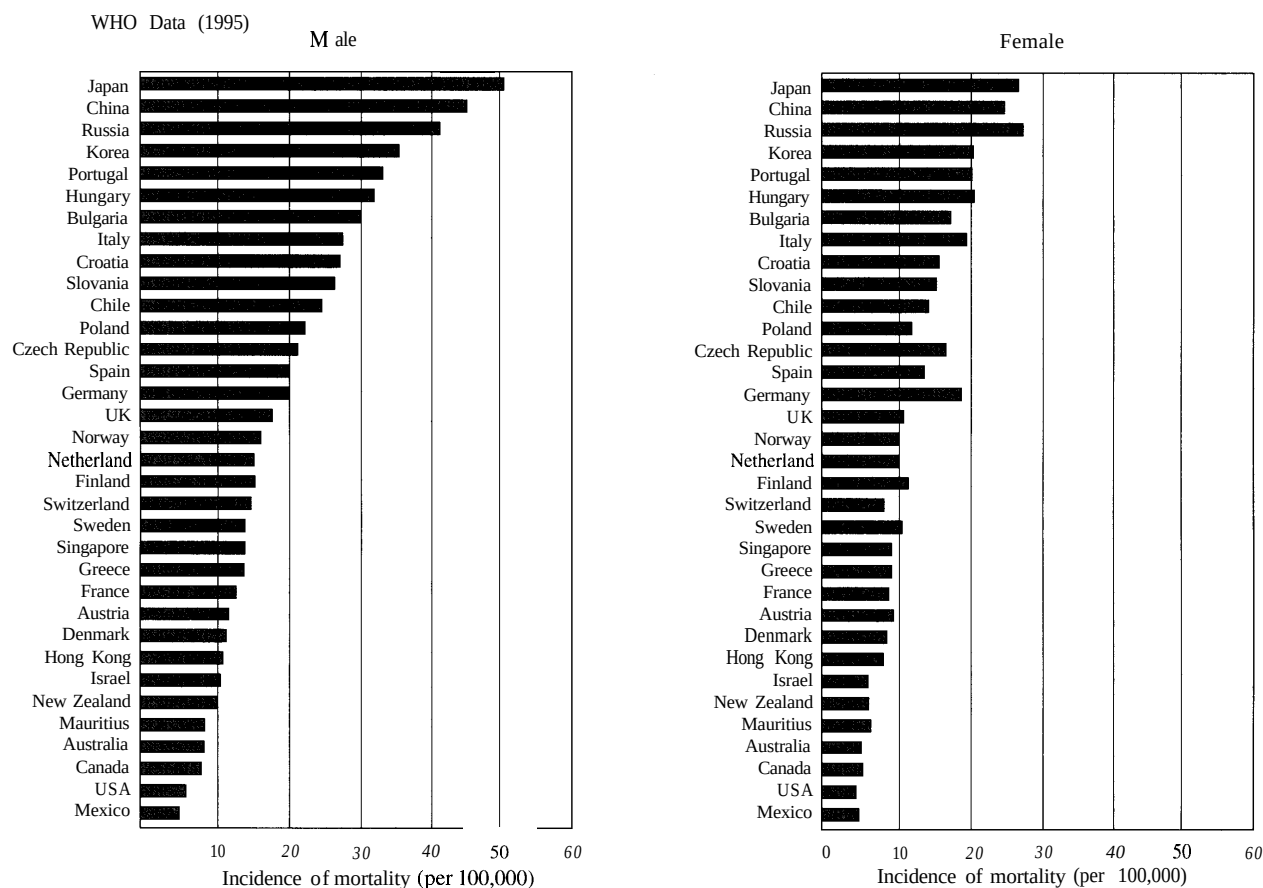


Fig. 1. Mortality of stomach cancer based on World Health Organization data. Note that the rates in males are consistently higher than those in females by about 2-fold.

WHO Data (1997)

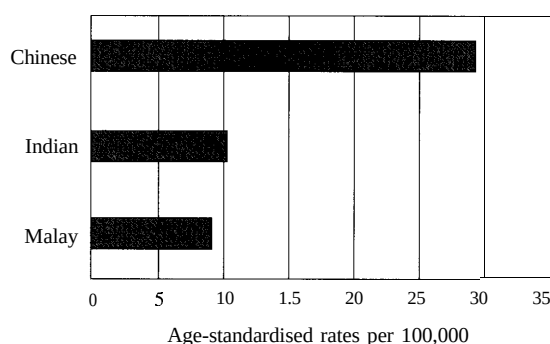


Fig. 2. Incidence of stomach cancer in Singaporean males of different ethnic groups. Age-standardized rate is the incidence rate that, theoretically, would have been observed if the population had the standard age distribution, using the world population as the reference population.

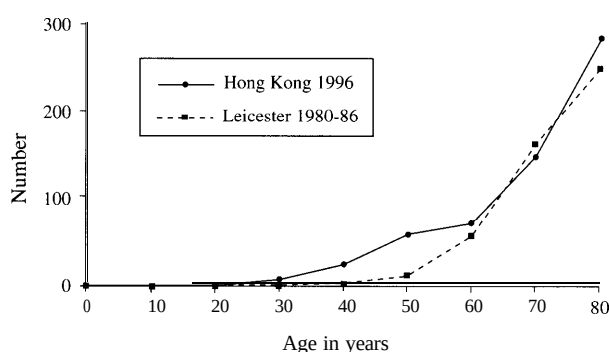


Fig. 3. Mortality of stomach cancer by age in Hong Kong, China and in Leicester, England showing a much earlier age of onset in the Chinese, a finding also well observed in Singapore and Japan.

above.¹⁵ In high risk regions like Japan, the incidence rates in 1981 increased from 82.8 per 100,000 men aged 45-49 years to 572.1 per 100,000 men aged 85 years or above.¹⁶

The age at which gastric cancer starts to appear is younger in East Asians, in whom the kick-off age is 30 years, compared with that in Western populations, in whom this is usually 50 years (Fig. 3). This has important bearing in the diagnostic approach to the problem of dyspepsia in East Asians.¹⁷

The male to female ratio in incidence rate of gastric cancer is usually around 1.5:3.0 worldwide, while in China it varies from 1.6:3.9.¹⁸ It should be noted that there is no sex difference in the prevalence of *H. pylori*.

Disease Pattern

The diffuse and intestinal types of gastric cancer as classified by Lauren described two biological entities that are different with regards to epidemiology, aetiology, pathogenesis and behaviour. While there was a worldwide decline in the incidence of the intestinal type in the recent few decades as parallel to the overall incidence of gastric cancer, a gradual increase in the

diffuse type was observed, which now accounts for approximately 30% of gastric carcinoma in some reported series.¹⁹

An explosive increase in incidence of gastric cancers confined to the cardia has been observed.²⁰⁻²² The shift from distal to proximal stomach was partially contributed by the decrease in distal cancers. Proximal tumours share demographic and pathological features with Barrett's associated oesophageal adenocarcinoma and are more likely to occur in men, and this parallels the male predominance in the increasing incidence of lower third oesophageal carcinoma. Proximal tumours differ from distal tumours in that they are not associated with a severe form of gastritis characterised by atrophy and/or intestinal metaplasia. They tend to be more aggressive than those arising from the distal sites. It has been proposed that environmental factors or chemical carcinogens, e.g. cigarette and alcohol, have been particularly associated with cardia carcinoma. In fact, it has been proposed that carcinoma of the cardia is a different entity from that of the rest of the gastric carcinoma.

Aetiology

The association of gastric cancer with blood group A has led to the suggestion of a genetic component to its pathogenesis as early as 1953.²³ However, the progress of understanding of the genetic basis of gastric cancer has picked up speed only in the last decade, with the advent of molecular medicine. A recent interesting study in a family in New Zealand with gastric cancer has demonstrated a mutation in the cadherin gene, which caused the cancer to develop, has shone some light on the understanding of the genetic pathogenesis of gastric cancer.²⁴

Helicobacter pylori

The dramatic variations in the incidence of gastric cancer in different geographic areas and from one generation to the next have led to the hypothesis that the incidence of gastric cancer is determined largely by environmental rather than genetic factors. Environmental factors such as dietary and socio-economical changes have been implicated.²⁵ Recently there has been growing interest in *H. pylori* infection as a potential carcinogen.²⁶ The sequence of infection by *H. pylori* to cancer is presumed to be due to the development of acute gastritis, followed by progression to chronic gastritis, intestinal metaplasia, dysplasia and adenocarcinoma.²⁷⁻²⁹ However, this hypothesis is mainly based on the result of epidemiological studies. Large chemoprevention studies by eradicating *H. pylori* infection are in progress.³⁰ Nevertheless, whatever the carcinogen is, the final pathway for the causation of cancer will be changes at the molecular level. In fact, several recent studies have shown a relationship between *H. pylori* and the molecu-

lar changes from precancerous to the cancerous stage.³¹⁻³⁷ Molecular studies may give a definite proof of the aetiological role of *H. pylori* in inducing gastric carcinoma.

Dietary Risk Factors

Salt

Over sixteen case-control studies on salt or salted food showed varying results. Statistically significant increase in risk was seen in both areas with low gastric cancer incidence, like USA (OR = 3.1),³⁸ and areas with high gastric cancer incidence, like Korea (OR = 3.7).³⁹ Other studies showed no substantial association in salt intake in both areas with low gastric cancer incidence, like USA (OR = 1.2)⁴⁰ and areas with high gastric cancer incidence, like China (OR = 1.1).⁴¹ Similar results were seen in studies looking at the use of table salt. Three studies from countries with varying gastric cancer incidence showed statistically significant increase in risk (OR = 1.6-6.2),⁴²⁻⁴⁴ while other studies showed no significant or no association.⁴⁵⁻⁴⁸ The Intersalt study involving 24 countries showed a correlation between 24 h urinary sodium excretion and stomach cancer mortality ($r = 0.70$ in men and 0.74 in women).⁴⁹ Both countries with high stomach cancer mortality, such as China and Japan, as well as with low stomach cancer mortality, such as England and Iceland were included. However, within individual countries, results were not consistent. Studies in Japan showed that geographical variation in stomach cancer mortality did not correlate with per capita salt consumption.^{50,51} In China, on the other hand, salt-preserved vegetables were weakly associated with stomach cancer mortality in 65 rural counties.⁵² The same study also showed that salt consumption and soy sauce consumption were related to elevated risk of dysplasia, a premalignant lesion, to gastric cancer. Our preliminary analysis based on a separate study in China also suggested that salted fish consumption increased the risk of gastric precancerous lesions.⁵³ Studies in Japan and Italy showed similar correlation between salted fish and premalignant lesions.^{54,55}

Salt, by itself, is not carcinogenic. The biological pathways of salt in stomach cancer have been described.⁵⁶ Intake of salt or salty food lead to damage of the mucosal protective layer of the stomach and increased DNA synthesis and cell proliferation.^{57,58} This can potentiate the action of carcinogens and increases the possibility of increased rate of endogenous mutations. An excessive salt intake can also induce atrophy in experimental animals and in human gastric mucosa.^{59,60}

Nitrates, Nitrites and N-nitroso Compounds

The belief that N-nitrosated compounds are carcinogenic came mostly from animal data. There is extensive evidence that they are carcinogenic to the stomach as

well as other organs in animals.⁶¹ Nitrates occur naturally in plants, but can be higher in the presence of nitrate fertilizers. Nitrate is found largely in food as it is used as a preservative in 'cured' food. Nitrates and nitrites are not carcinogenic in animals. Ingestion of nitrates from food may be reduced bacterially to nitrites with subsequent nitrosation. Amino substrates present in food, drugs, and other sources may interact with nitrite under appropriate conditions to form N-nitroso compounds, many of which are carcinogens in animals.⁶² Those which do not require metabolic activation, such as N-nitrosamides, could be locally acting in the stomach. Inhabitants in high-risk areas for gastric cancer had significantly higher exposure to endogenous N-nitroso compounds.⁶³ However, seven case control studies examining intake of nitrate, nitrite and nitrosamines drew no conclusion for each compound.^{40,45-47,64-66} Analysis of 15 correlation studies on variable measures of nitrate exposure to gastric cancer in 10 countries showed that one-third demonstrated increased risk, one third showed decreased risk and one third showed no association.⁶⁷

Other confounding factors in the diet may affect the theoretical risk of dietary N-nitroso compounds. Concomitant intake of vitamin C or α -tocopherol in vegetables may counteract the increased risk of nitrates inside. In the Intersalt study, correlation of high urinary nitrate excretion was found in countries with high salt intake. In countries with low salt intake, higher nitrate levels were associated with lower mortality.⁴⁹ Association between nitrite-cured food and stomach cancer may also be due to other preservation method and materials.^{45,47} In conclusion, there is probably no relation between nitrates in vegetables and gastric cancer. The possible role of N-nitroso compound in stomach carcinogenesis requires more supporting data.

Dietary Preventive Factors

Vegetables and fruits

Consumption of vegetables and fruits is most widely studied in relation to gastric cancer. Among all cancers, lung and stomach cancer are the two sites for which most of the prospective data are available. Most of the prospective cohort studies showed a relative risk of 0.5 to 0.9⁶⁸⁻⁷³ and case-control studies showed an odds ratio from 0.1 to 0.8.^{38-41,44,45,47,48,66,69,74-77} The evidence for raw vegetables, allium vegetables and citrus fruits are convincing. But salted and pickled vegetables are usually associated with inconsistent results. Studies on precancerous lesions have shown protective effect of fruits and vegetables in USA, China and Colombia.^{52,53,75,78}

Micronutrients and Minerals

Carotenoids

High intake of beta-carotene has been associated with a decreased risk in six out of eight case-control studies in

both areas of high and low gastric cancer mortality.^{41,45,47,48,79,80} A dose-response inverse relationship between serum beta-carotene and prevalence of chronic atrophic gastritis was seen.⁸¹ In China, a significant inverse association with intake of green vegetables and levels of plasma selenium and beta-carotene was observed⁵² and people with intestinal metaplasia or dysplasia had serum carotene levels about 10% lower than those without these conditions.⁸² Carotene is probably protective through its potent anti-oxidant effect.

Vitamin C

Most prospective and case-control studies have found a significant protective effect of dietary intake of vitamin C.^{38,40,41,44,45,47,48,80,83} A geographical correlation study in China has reported a weak inverse correlation between serum vitamin C levels and stomach cancer mortality in 65 counties.⁵² One case-controlled study of chronic atrophic gastritis showed reduced risk with higher vitamin C intake.⁷⁸ Although there was no association between serum vitamin C levels and gastric precancerous lesions in Colombia,⁸⁴ serum vitamin C levels were significantly lower in those patients with intestinal metaplasia or dysplasia than those with superficial gastritis or chronic atrophic gastritis in China.⁸²

Tea

The relationship between tea drinking and stomach cancer has been studied in three cohort studies and 12 case-control studies, and has been reviewed by Kohlmeier et al.⁸⁵ Overall, these epidemiologic studies gave no conclusive evidence of a protective effect of tea on gastric cancer. This could be due to the different kinds of tea, mainly green and black tea, which have different constituents and potentially different biological effects. Looking at green tea separately, there was a possible inverse relationship between green tea and the risk of gastric cancer. Four of five case-control studies suggested a protective effect.^{50,68,76,86,87} The protective mechanism remains unknown. One possible mechanism involved the inhibition of the formation of N-nitroso compounds by the polyphenols of tea. Polyphenol extracts of green tea are known to have anticarcinogenic effects in animals.⁸⁸ An animal study showed that epigallocatechin, a principal component of green tea polyphenols, can inhibit chemical carcinogenesis in the stomach.⁸⁹ For black tea, most of the case-control studies and cohort studies showed no association with the risk of stomach cancer.^{47,66,76,77} It has been suggested that the anticarcinogenic effects of green tea catechins may be stronger than those of black tea polyphenols.⁹⁰

An expert panel report on food and nutrition and cancer, commissioned by the World Cancer Research Fund and the American Institute for Cancer Research, suggested that vegetables and fruits and the use of

refrigeration has shown convincing evidence for decreasing gastric cancer.⁹¹ Vitamin C, contained in vegetables and fruits and other foods of plant origin, is probably protective. Salt in diet and salting of food probably increased the risk of gastric cancer. Carotenoids, allium compounds, and green tea showed possible evidence of reducing the risk, while diets high in starch, grilled meat and fish, possibly increased the risk. Alcohol, coffee, black tea, nitrates, vitamin E, retinol and sugar possibly had no relation with gastric cancer. There is insufficient data to conclude about fibre, selenium and garlic (protective), cured meat, and N-nitrosamines (carcinogenic), in the risk of gastric cancer.⁹¹ The panel judged that diet high in a variety of vegetables and fruits, together with the use of freezing and refrigeration to preserve perishable food, and a low consumption of salt and salted food may prevent 66% to 75% of the cases of gastric cancer.

Genetic Model for Gastric Tumourigenesis

The rapid progress in the understanding of molecular biology of cancer has provided increasing evidence that transformation from a normal epithelial cell to a malignant cell is a multistep process and results from an accumulation of multiple gene abnormalities. The hereditary and non-hereditary colon cancer serves as a well-known model for the study of molecular pathogenesis in gastric cancer. It clearly demonstrates the stepwise proliferative sequence of the colonic epithelium to colonic cancer, with different genetic alterations being detected in different stages.⁹²

Gastric cancer is an interesting model for studying tumourigenesis because it represents a heterogeneous condition. The Lauren classification,⁹³ which differentiates the gastric carcinoma into diffuse and intestinal types, has been the most widely used because it describes two biological entities that are different in epidemiology, aetiology, pathogenesis and behaviour. Not surprisingly, the molecular genetic studies in gastric carcinogenesis have shown that the development of intestinal type and diffuse type of gastric cancer follows two different pathways.

Progressive accumulations of genetic changes have been evidenced in the different stages in Correa's model. Microsatellite instability^{94,95} and telomerase reactivation⁹⁶ occurred in early carcinogenesis. Mutation of p53,⁹⁷ K-ras⁹⁸ and adenomatous polyposis coli (APC) genes,⁹⁹ amplification and overexpression of c-myc¹⁰⁰ and cyclin E genes¹⁰¹ progressively accumulate in the advanced gastric cancer. Multiple autocrine and paracrine loops interact with each other for the progression of advanced gastric cancer. These include hepatocyte growth factor¹⁰² and c-met gene,¹⁰³ fibroblast growth factor and K-sam gene,¹⁰⁴ epidermal growth factor and c-erb-2, HER-2/neu genes¹⁰⁵⁻¹⁰⁹ EGF /TGF α and EGF receptors.¹⁰⁹ E-

cadherin^{110,111} CD44 and matrix metalloproteinases¹¹² have been shown to play an important role in metastasis.

Helicobacter pylori

The relationship between *H. pylori* and molecular changes may be another way to study the role of *H. pylori* in gastric carcinogenesis. Kuniyasu et al³⁵ found that the degree of *H. pylori* infection correlated with the level of human telomerase RNA expression (hTR) and telomerase positivity in 26 carcinoma tissues. Moss et al³¹ showed that apoptotic cells occurred in about 2.9% of epithelial cells in uninfected gastric tissue samples, located in the most superficial aspect of the gastric glands. Whereas apoptotic cells were found in 16.8% in infected gastric tissues throughout the depth of gastric glands, the value fell to 3.1% after *H. pylori* eradication.³¹ Similar results have been obtained by others.^{32,33} Accumulation of mutant p53 protein at the regenerating zone of gastric pits was significantly decreased in the *H. pylori* eradicated group than the non-eradicated group.¹¹³ Downregulation of the E-cadherin protein has been shown to be significantly associated with *H. pylori* infection in patients with normal gastric mucosa, gastritis, gastric ulcer and duodenal ulcer.³⁴ Studies on whether eradication of *H. pylori* results in reversion or halting of the molecular events are important both in the understanding of gastric carcinogenesis as well as in the management of patients.

Intestinal versus Diffuse Types of Gastric Carcinoma

Genetic differences have been observed in intestinal and diffuse types, suggesting two different pathways of carcinogenesis (Table I). Microsatellite instability is found in 64% in diffuse type but only 17% in intestinal type.¹¹⁴ Mutations of p53 gene are essentially restricted to the intestinal type in the early phase, but involved both types in the advanced stage.^{115,116} LOH and mutation of the APC gene are frequently associated with intestinal type gastric cancers, observed in more than 50% in one series¹¹⁷ but seldom found in diffuse type. The deletion in colon cancer (DCC) gene, which was located on chromosome 18q, was frequently observed in 50% to 61% of intestinal type but not in diffuse type.¹¹⁸

While c-erb-2 amplification is associated with intestinal type adenocarcinoma, amplifications of c-met and k-sam tyrosine kinase receptor genes are frequently found in diffuse type.¹⁰⁴ The diffuse type carcinomas correlate with overexpression of EGF family, TGF β , platelet-derived growth factor (PDGF), insulin-like growth factor (IGF-II) and fibroblast growth factor (FGF).¹⁰⁹

The involvement of the cadherin gene seems to be one of the most important steps in diffuse type carcinogenesis and takes place at an early stage. Decreased expression of E-cadherin by immunohistochemical staining has been found in most of the diffuse type gastric cancer. The cadherin gene has been called the invasion suppres-

TABLE I: GENETIC ALTERATIONS IN THE INTESTINAL AND DIFFUSE TYPES OF GASTRIC CANCER

Genes (alteration)	Diffuse (%)	Intestinal (%)
Genetic instability	40	11
LOH and tumour suppressor genes:		
LOH of 1p	38	25
LOH of 1q		44
LOH of 7q	33	53
P53 (LOH, mutation)	76	60
APC (LOH, mutation)		40-60
DCC (LOH)		50
Activation of protooncogenes:		
K-sam (amplification)	33	
Tyrosine kinase receptor genes:		
c-met: amplification	39	19
6.0 kb mRNA	82	50
K-ras: mutation		9
C-erb-2: amplification		20
Bcl-2 (LOH)		43
Cyclin E (amplification)	10	10
Metastasis-related genes:		
Cadherin, catenin (deletion)	50	
CD44 (abnormal transcript)	100	100

sor gene and plays an important role in the carcinogenesis of the diffuse type carcinoma which is characterised by diffuse infiltration and high invasion potential. Mutation of the cadherin gene has been found to be the cause of familial carcinoma of stomach as mentioned above.²⁴

Conclusion

Despite the decline in incidence, gastric carcinoma still carries the second highest cancer mortality in the world. The development of molecular biology may be used as prognostic markers for identifying high risk patients who requires more aggressive treatment; as well as developing innovative therapeutic targets. For example, the study of EGF receptor antagonist is underway which might become a powerful therapeutic agent. The study of thymidilate synthase gene expression may help to identify patients who are more likely to benefit from chemotherapy.

However, in addition to searching for better treatment modalities, both primary and secondary prevention of gastric cancer should be aimed at. Mass screening by endoscopy has already achieved success in Japan, which accounts for the better survival rates in the Japanese. The use of molecular genetics, such as microsatellite instability, may be a potentially valuable tool for identifying patients at risk for developing gastric cancer. The effectiveness of diet modification and the primary prevention by *H. pylori* chemo-eradication and vaccination are currently being evaluated as well. We look forward to a gastric cancer-free world in the next century.

REFERENCES

- Haenszel W. Variation in incidence of and mortality from stomach cancer, with particular reference to the United States. *J Natl Cancer Inst* 1958; 21:213-62.
- Munoz N, Asvall J. Time trends of intestinal and diffuse types of gastric cancer in Norway. *Int J Cancer* 1971; 8:144-57.
- Hirayama T. Epidemiology of cancer of the stomach with special reference to its recent decrease in Japan. *Cancer Res* 1975; 35:3460-3.
- Piper D W. Stomach cancer. Geneva: International Union Against Cancer. UICC Technical Report Series, Vol 34, 1978.
- Waterhouse J, Muir C, Correa I', et al, editors. Cancer incidence in five continents. Vol III. IARC Scientific Publication no.15. Lyon: International Agency for Research on Cancer, 1976.
- WHO. Cancer Incident in Five Continents. Vol 7. World Health Organization, 1997.
- Chen J S, Campbell T C, Li JY, Peto R. Diet, Life-style and Mortality in China. A Study of the Characteristics of 65 Chinese Counties. Oxford: Oxford Univ Press, 1990.
- Wong BCY, Ching CK, Lam SK, Li ZL, Chen BW, Li YN, et al. Differential north to south gastric cancer-duodenal ulcer gradient in China. *J Gastroenterol Hepatol* 1998; 13:1050-7.
- Wynder E L, Kmet J, Dungall N, et al. An epidemiologic investigation of gastric cancer. *Cancer* 1963; 16:1461-96.
- Correa P, Haenszel W, Tannenbaum S. Epidemiology of gastric carcinoma: review and future prospects. *Natl Cancer Inst Monogr* 1982; 62:129-34.
- Asaka M, Kato M, Kudo M, et al. Relationship between *Helicobacter pylori* infection, atrophic gastritis and gastric carcinoma in a Japanese population. *Eur J Gastroenterol Hepatol* 1995; 7(suppl 1):7-10.
- Correa P, Cuello C, Duque E. Carcinoma and intestinal metaplasia of the stomach in Colombian migrants. *J Natl Cancer Inst* 1970; 44:297-306.
- Cuello C, Correa P, Haenszel W, et al. Gastric cancer in Columbia. I. Cancer risk and suspect environmental agents. *J Natl Cancer Inst* 1976; 57:1015-20.
- Statistics and Information Department, Minister's Secretariat, Ministry of Health and Welfare of Japan: Age-adjusted Death Rates by Prefecture. Special Report on Vital Statistics 1990. Tokyo, Japan: Kosei Tokkei Kyokai, 1992.
- Ries LA, Hankey B F, Edwards B K, editors. Cancer Statistics Review 1973-1987. US Department of Health and Human Services, Public Health Service, NIH Publication 90-2789, Washington DC: Government Printing Office, 1990.
- Muir C, Waterhouse J, Mack T, Powell J, Whelan S, editors. Cancer in five continents. Vol V. Lyon: (IARC) Scientific Publications No. 88, 1987.
- Lam S K, Talley NJ. *Helicobacter pylori* Consensus: Report of the 1997 Asia Pacific Consensus Conference on the Management of *Helicobacter pylori* Infection. *J Gastroenterol Hepatol* 1998; 13:1-12.
- Ching CK, Lam SK. *Helicobacter pylori* epidemiology in relation to peptic ulcer and gastric cancer in south and north China. *J Gastroenterol Hepatol* 1994; 9(Suppl 1):S4-7.
- Ideda Y, Mori M, Kamakura T, Haraguchi Y, Saku M, Sugimachi K. Improvements in diagnosis have changed the incidence of histological types in advanced gastric cancer. *Br J Cancer* 1995; 72:424.
20. Dupont J B, Lee J R, Burton G R, et al. Adenocarcinoma of the stomach: review of 1497 cases. *Cancer* 1978; 41:941-7.
- Kampschoer GH M, Nakajima T, Van De Velde C J H. Changing patterns in gastric adenocarcinoma. *Br J Surg* 1989; 76:914-6.
- Powell J, McConkey C C. Increasing incidence of adenocarcinoma of the gastric cardia and adjacent sites. *Br J Cancer* 1990; 62:440-3.
- Aird I, Bentall H, Roberts J F. Relationship between cancer of stomach and the ABO blood groups. *Br Med J* 1953; 1:799-801.
24. Guilford I', Hopkins J, Harraway J, et al. E-cadherin germline mutations in familial gastric cancer. *Nature* 1998; 392:402-5.
- Bartsch H, O'Neill I, Hermann R. The relevance of N-nitroso compounds to human cancer. Exposures and mechanisms: IARC Scientific Publications No 84. Lyon, France: International Agency for Research on Cancer, 1987; 84:1-663.
26. Parsonnet J, Friedman G D, Vandersteen D P, et al. *Helicobacter pylori* infection and the risk of gastric carcinoma. *N Engl J Med* 1991; 325:1127-31.
27. Correa P, Haenszel W, Cuello C, Arder M, Tannenbaum S R. A model for gastric cancer epidemiology. *Lancet* 1975; 2:58-60.
28. Correa I'. The gastric precancerous process. *Cancer Surv* 1983; 2:437-50.
29. Correa P. A human model of gastric carcinogenesis. *Cancer Res* 1988; 48:3554-60.
30. Forman D. Lessons from ongoing intervention studies. In: Hunt R H, Tytgat G N J, editors. *Helicobacter pylori*: Basic Mechanisms to Clinical Care, 1998. Dordrecht: Kluwer Academic Publishers, 1998:354-60.
31. Moss S F, Calam J, Agarwal B, Wang S, Holt P G. Induction of gastric epithelial apoptosis by *Helicobacter pylori*. *Gut* 1996; 38:498-501.
32. Jones N L, Yeager H, Cutz E, Sherman P M. *Helicobacter pylori* induces apoptosis of gastric antral epithelial cells in vivo. *Gastroenterology* 1996; 110:A933.
33. Bravo L E, Mannick E E, Zhang X J, Ruiz B, Correa I', Miller M J S. *H. pylori* infection is associated with inducible nitric oxide synthase expression, nitrotyrosine and DNA damage. *Gastroenterology* 1995; 108:A63.
34. Terres A M, Pajares J M, O'Toole D, Ahern S, Kelleher D. *H. pylori* infection is associated with downregulation of E-cadherin, a molecule involved in epithelial cell adhesion and proliferation control. *J Clin Pathol* 1998; 51:410-2.
35. Kuniyasu H, Domen T, Hamamoto T, et al. Expression of human telomerase RNA is an early event of stomach carcinogenesis. *Jpn J Cancer Res* 1997; 88:103-7.
36. Lin S T, Wu M S, Shun C T, et al. Microsatellite instability in gastric carcinoma with special references to histopathology and cancer stages. *Eur J Cancer* 1995; 31A:1879-82.
37. Wu M S, Shun C T, Wang H P, et al. Genetic alterations in gastric cancer: relation to histological subtypes, tumour stage, and *Helicobacter pylori* infection. *Gastroenterology* 1997; 112:1457-65.
38. Graham S, Haughey B, Marshall J, Brasure J, Zielezny M, Freudenheim J, et al. Diet in the epidemiology of gastric cancer. *Nutr Cancer* 1990; 13:19-34.
39. Lee J K, Park B J, Yoo K Y, Ahn Y O. Dietary factors and stomach cancer: a case-control study in Korea. *Int J Epidemiol* 1995; 24:33-41.
40. Correa I', Fontham E, Pickle L W, Chen V, Lin Y, Haenszel W. Dietary determinants of gastric cancer in south Louisiana inhabitants. *J Natl Cancer Inst* 1985; 75:645-54.
41. You W C, Blot W J, Chang Y S, Ershow A G, Yang Z, An Q, et al. Diet and high risk of stomach cancer in Shangdong, China. *Cancer Res* 1988; 48:3518-23.
42. Tuyns A J. Salt and gastrointestinal cancer. *Nutr Cancer* 1988; 11:229-32.
43. Coggon D, Barker D J I', Cole R B, Nelson M. Stomach cancer and food storage. *J Natl Cancer Inst* 1989; 81:1178-82.
44. Boeing H, Jedrychowski, Wahrendorf J, Popiela T, Tobiasz-Adamczyk B, Kulig A. Dietary risk factors in intestinal and diffuse types of stomach cancer: a multicenter case-control study in Poland. *Cancer Causes Control* 1991; 2:227-33.
45. Buiatti E, Palli D, Decarli A, Amadori D, Avellini C, Bianchi S, et al. Case-control study of gastric cancer and diet in Italy. *Int J Cancer* 1989; 44:611-6.
46. Boeing H, Frentzel-Beyme R, Berger M, Berndt V, Gores W, Korner M, et al. Case-control study on stomach cancer in Germany. *Int J Cancer* 1991; 47:858.
47. Risch H A, Jain M, Choi N W, Fodor J G, Pfeiffer C J, Howe G R, et al. Dietary factors and incidence of cancer of the stomach. *Am J Epidemiol* 1985; 122:947.
48. La Vecchia C, Negri E, Decarli A, Avanzo B D, Franceschi S A. A case-control study of diet and gastric cancer in northern Italy. *Int J Cancer* 1987; 40:484-9.
49. Joossens J V, Hill M J, Elliott P, Stamler R, Stamler J, Lesaffre E, et al. Dietary salt, nitrate and stomach cancer mortality in 24 countries. *Int J Epidemiol* 1996; 25:494-504.
50. Kono S, Ikeda M, Ogata M. Salt and geographical mortality of gastric cancer and stroke in Japan. *J Epidemiol Community Health* 1983; 37:43-6.

51. Honjo S, Kono S, Yamaguchi M. Salt and geographical variation in stomach cancer mortality in Japan. *Cancer Causes Control* 1994; 5:285-6.
52. Kneller R W, Guo W D, Hsing A W. Risk factors for stomach cancer in sixty-five Chinese counties. *Cancer Epidemiol Biomarkers Prev* 1992; 1:113-8.
53. Chen J S, Wong B C Y, Wu Y N, Lin H, Wang J L, Ho J, et al. Preliminary analysis of dietary risk factors for precancerous gastric lesions in population of Changle, a high risk area of gastric cancer in China. *Gastroenterology* 1997; 112:A547.
54. Nomura A, Yamakawa H, Ishidate T, Kamiyama S, Masuda H, Stemmermann G N, et al. Intestinal metaplasia in Japan: association with diet. *J Natl Cancer Inst* 1982;68:401-5.
55. The ECP-EURONUT-IM study group. Serum selenium concentrations in patients with intestinal metaplasia and in controls. *Eur J Cancer Prev* 1992; 1:31-4.
56. Correa P. Human gastric carcinogenesis: a multistep and multifactorial process-First American Cancer Society Award Lecture on Cancer Epidemiology and Prevention. *Cancer Res* 1992; 52:6735-40.
57. Charnley G, Tannenbaum S R. Flow cytometric analysis of the effect of sodium chloride on gastric cancer risk in the rat. *Cancer Res* 1985; 45.
58. Ames BN, Gold L S. Too many rodent carcinogens: mitogenesis increases mutagenesis. *Science* 1990; 249:970-1.
59. Kodama M, Kodama T, Susuki H, Kondo K. Effect of rice and salty rice diet on the structure of mouse stomach. *Nutr Cancer* 1984; 6:135-47.
60. Correa P, Haenszel W, Cuello C, Arder M, Tannenbaum S R. A model for gastric cancer epidemiology. *Lancet* 1975; 2:58-60.
61. Mirvish S S. The etiology of gastric cancer :intragastric nitrosamide formation and other theories. *J Natl Cancer Inst* 1983; 71:629-47.
62. Preussman R, Stewart B. N-nitroso carcinogens. In: Searle C, editor. *Chemical Carcinogens*. Washington DC: American Chemical Society ACS Monograph 1984; 182:643-828.
63. Kamiyama S, Michioka O, Simada A, Miura K. Mutagenic components of diet and epidemiology of stomach cancer. *Gan-no-Rinsho* 1986; 32:699-706.
64. Palli D, Bianchi S, Decarli A, Cipriani F, Avellini C, Cocco P, et al. A case-control study of cancers of the gastric cardia in Italy. *Int J Cancer* 1992; 65:263-6.
65. Gonzalez C A, Riboli E, Badosa J, Batiste E, Cardona T, Pita S, et al. Nutritional factors and gastric cancer in Spain. *Am J Epidemiol* 1994; 139:466-73.
66. Hansson L E, Nyren O, Bergstrom R, Wolk A, Lindgren A, Baron J, et al. Nutrients and gastric cancer risk. A population-based case-control study in Sweden. *Int J Cancer* 1994; 57:638-44.
67. Forman D. Are nitrates a significant risk factor in human cancer? *Cancer Surv* 1989; 8:443-58.
68. Kato I, Tominaga S, Matsumoto K. A prospective study of stomach cancer among a rural Japanese population: a 6-year survey. *Jpn J Cancer Res* 1992; 83:568-75.
69. Hirayama T. A large-scale study on cancer risks by diet-with special reference to the risk reducing effects of green-yellow vegetable consumption. In: Hayashi Y, Magao M, Sugimura T, et al, editors. *Diet, Nutrition and Cancer*. Tokyo: Japan Scientific Societies Press, 1986:41-53.
70. Kneller R W, McLaughlin J K, Bjelke E, Schuman L M, Blot W J, Wacholder S, et al. A cohort study of stomach cancer in a high-risk American population. *Cancer* 1991; 68:672-8.
71. Chyou P H, Nomura A M Y, Hankin J H, Stemmermann G N. A case-control study of diet and stomach cancer. *Cancer Res* 1990; 50:7501-4.
72. Nomura A M, Stemmermann G N, Chyou P H. Gastric cancer among the Japanese in Hawaii. *Jpn J Cancer Res* 1995; 86:916-23.
73. Dorant E, Van den Brabdt P A, Goldbohm R A, Sturmans F. Consumption of onions and a reduced risk of stomach carcinoma. *Gastroenterology* 1996; 110:12-20.
74. Wong B C Y, Lam S K. Diet and gastric cancer. *Gastrointest Cancer* 1998; 3:1-10.
75. Haenszel W, Kurihara M, Locke F B, Shimizu K, Segi M. Stomach cancer in Japan. *J Natl Cancer Inst* 1976; 56:265-78.
76. Tajima K, Tominaga S. Dietary habits and gastrointestinal cancers: a comparative case-control study of stomach and large intestinal cancers in Nagoya, Japan. *Jpn J Cancer Res* 1985; 76:705-16.
77. Demirel T, Icli F, Uzunalimoglu O, Kucuk O. Diet and stomach cancer incidence: a case-control study in Turkey. *Cancer* 1990; 65:2344-8.
78. Fontham E, Zavala D, Correa P, Rodriguez E, Hunter F, Haenszel W, et al. Diet and chronic atrophic gastritis: a case-control study. *J Natl Cancer Inst* 1986; 76:621-7.
79. Hansson L E, Nyren O, Bergstrom R, Wolk A, Lindgren A, Baron J, et al. Nutrients and gastric cancer risk. A population-based case-control study in Sweden. *Int J Cancer* 1994; 57:638-44.
80. Ramon J M, Serra L, Cerdo C, Oromi J. Dietary factors and gastric cancer risk: a case-control study in Spain 1993; 71:1731-5.
81. Tsugane S, Kabuto M, Imai H, et al. *Helicobacter pylori*, dietary factors and atrophic gastritis in five Japanese populations with different gastric cancer mortality. *Cancer Causes Control* 1993; 4:297-305.
82. Zhang L, Blot W J, You W C, Chang Y S, Liu X Q, Kneller R W, et al. Serum micronutrients in relation to precancerous gastric lesions. *Int J Cancer* 1994; 56:650-4.
83. Gonzalez C A, Riboli E, Badosa J, Batiste E, Cardona T, Pita S, et al. Nutritional factors and gastric cancer in Spain. *Am J Epidemiol* 1994; 139:466-73.
84. Haenszel W, Correa I, Lopez A, Cuello C, Zarama G, Zavala D, et al. Serum micronutrient levels in relation to gastric pathology. *Int J Cancer* 1985; 36:43-8.
85. Kohlmeier L, Weterings K G C, Steck S, Kok F J. Tea and Cancer prevention: an evaluation of the epidemiologic literature. *Nutr Cancer* 1997; 27:1-13.
86. Yu G I, Hsieh C C. Risk factors for stomach cancer: a population-based case-control study in Shanghai. *Cancer Causes Control* 1991; 2:169-74.
87. Yu G P, Hsieh C C, Wang L Y, Yu S Z, Li X L, Jin T H. Green-tea consumption and risk of stomach cancer: a population-based case-control study in Shanghai, China. *Cancer Causes Control* 1995; 6:532-8.
88. Yang C S, Wang Z Y. Tea and cancer. *J Natl Cancer Inst* 1993; 85:1038-49.
89. Yamane T, Takahashi T, Kuwata K, Oya K, Inagake M, Kitao Y, et al. Inhibition of N-methyl-N'-nitro-N-nitrosoguanidine-induced carcinogenesis by (-)-epigallocatechin gallate in the rat glandular stomach. *Cancer Res* 1995; 55:2081-4.
90. Wang Z Y, Hong J Y, Huang M T, Reuhl K R, Conney A H, Yang Y S. Inhibition of N-nitrosodiethylamine- and 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone induced tumorigenesis in A/J mice by green tea and black tea. *Cancer Res* 1992; 52:1943-7.
91. Food, Nutrition and the Prevention of Cancer: a Global Perspective. Washington, USA: World Cancer Research Fund / American Institute for Cancer Research, 1997.
92. Fearon E R, Vogelstein B. A genetic model for colorectal tumorigenesis. *Cell* 1990; 61:759-67.
93. Lauren P. The two histologic main types of gastric carcinoma diffuse and so called intestinal type carcinoma. *Acta Pathol Microbiol Scand* 1965; 64:31-49.
94. Strickler J G, Zheng J, Shu Q, Burgart L J, Alberts S R, Shibata D. P53 mutations and microsatellite instability in sporadic gastric cancer: when guardians fail. *Cancer Res* 1994; 54:4750-5.
95. Semba S, Yokozaki H, Yamamoto S, Yasui W, Tahara E. Microsatellite instability in precancerous lesions and adenocarcinomas of the stomach. *Cancer* 1996; 77:1620-7.
96. Tahara E, Kuniyasu H, Yasui W, Yokozaki H. Gene alterations in intestinal metaplasia and gastric cancer. *Eur J Gastroenterol Hepatol* 1994; 6(suppl 1):S97-S101.
97. Sano T, Tsujino T, Yoshida K, et al. Frequent loss of heterozygosity on chromosomes 1q, 5q, and 17p in human gastric carcinomas. *Cancer Res* 1991; 51:2926-31.
98. Nanus D M, Kelsen D P, Mentle I R, Altorki N, Albino A I. Infrequent point mutations of ras oncogenes in gastric cancers. *Gastroenterology* 1990; 98:955-60.
99. Nakatsuru S, Yanagisawa A, Ichii S, et al. Somatic mutation of the APC gene in gastric cancer: frequent mutations in very well differentiated adenocarcinoma and signet-ring cell carcinoma. *Hum Mol Genet*

- 1992; 1:559-63.
100. Shibuya M, Yokota J, Ueyama Y. Amplification and expression of a cellular oncogene (c-myc) in human gastric adenocarcinoma cells. *Mol Cell Biol* 1985; 5:414-8.
 101. Akama Y, Yasui W, Yokozaki H, et al. Frequent amplification of the cyclin E gene in human gastric carcinomas. *Jpn J Cancer Res* 1995; 86:617-21.
 102. Taniguchi T, Kitamura M, Iwasaki Y, Yamamoto Y, Igari A, Toi M. Increase in the circulating level of hepatocyte growth factor in gastric cancer patients. *Br J Cancer* 1997; 75:673-7.
 103. Kuniyasu H, Domen T, Hamamoto T, et al. Expression of human telomerase RNA is an early event of stomach carcinogenesis. *Jpn J Cancer Res* 1997; 88:103-7.
 104. Tahara E. Molecular mechanism of stomach carcinogenesis. *J Cancer Res Clin Oncol* 1993; 119:265-72.
 105. Akiyama T, Sudo C, Ogawara H, Toyoshima K, Yamamoto T. The product of the human c-erbB-2 gene: a 185-kilodalton glycoprotein with tyrosine kinase activity. *Science* 1986; 232:1644-6.
 106. Coussens L, Yang-Feng T L, Liao Y C, et al. Tyrosine kinase receptor with extensive homology to EGF receptor shares chromosomal location with neu oncogene. *Science* 1985; 230:1132-39.
 107. Kameda T, Yasui W, Yoshida K, et al. Expression of ERBB2 in human gastric carcinomas: relationship between p185^{ERBB2} expression and the gene amplification. *Cancer Res* 1990; 50:8002-9.
 108. Tal M, Wetzler M, Josefsberg Z, et al. Sporadic amplification of the HER2/neu proto-oncogene in adenocarcinomas of various tissues. *Cancer Res* 1988; 48:1517-20.
 109. Tahara E. Growth factors and oncogenes in human gastrointestinal carcinoma. *J Cancer Res Clin Oncol* 1990; 116:121-31.
 110. Shimoyama Y, Hirohashi S. Expression of E- and P-cadherin in gastric carcinomas. *Cancer Res* 1991; 51:2185-92.
 111. Mayer B, Jauch K W, Gunthert U, et al. De-novo expression of CD44 and survival in gastric cancer. *Lancet* 1993; 342:1019-22.
 112. D'Errico A, Garbisa S, Liotta L A, Castronovo V, Stetler-Sterenson W G, Grigioni W F. Augmentation of type IV collagenase, laminin receptor, ki67 proliferative antigen associated with human colon, gastric, and breast carcinoma progression. *Mod Pathol* 1991; 4:239-46.
 113. Hibi K, Mitomi H, Koizumi W, Tanabe S, Saigenji K, Okayasu I. Enhanced cellular proliferation and p53 accumulation in gastric mucosa clinically infected with *Helicobacter pylori*. *Am J Clin Pathol* 1997; 108:26-34.
 114. Han H J, Yanagisawa A, Kato Y, Park J G, Nakamura Y. Genetic instability in pancreatic cancer and poorly differentiated type of gastric cancer. *Cancer Res* 1993; 53:5087-9.
 115. Uchino S, Noguchi M, Ochiai A, Saito T, Kobayashi M, Hirohashi S. I'53 mutation in gastric cancer: a genetic model for carcinogenesis is common to gastric and colorectal cancer. *Int J Cancer* 1993; 54:759-64.
 116. Ranzani G N, Luinetti O, Padovan L S, et al. P53 gene mutations and protein nuclear accumulation are early events in intestinal type gastric cancer but late events in diffuse type. *Cancer Epidemiol Biomarkers Prev* 1995; 4:223-31.
 117. Nakatsuru S, Yanagisawa A, Ichii S, et al. Somatic mutation of the APC gene in gastric cancer: frequent mutations in very well differentiated adenocarcinoma and signet-ring cell carcinoma. *Hum Mol Genet* 1992; 1:559-63.
 118. Yoshida Y, Itoh F, Endo T, Hinoda Y, Imai K. Decreased DCC mRNA expression in human gastric cancers is clinicopathologically significant. *Int J Cancer* 1998; 79:634-9.