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EDITORIAL

Carcinoma of the stomach: A review of epidemiology, pathogenesis, molecular genetics and chemoprevention

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Abstract

Carcinoma of the stomach is still the second most common cause of cancer death worldwide, although the incidence and mortality have fallen dramatically over the last 50 years in many regions. The incidence of gastric cancer varies in different parts of the world and among various ethnic groups. Despite advances in diagnosis and treatment, the 5-year survival rate of stomach cancer is only 20 per cent. Stomach cancer can be classified into intestinal and diffuse types based on epidemiological and clinicopathological features. The etiology of gastric cancer is multifactorial and includes both dietary and nondietary factors. The major diet-related risk factors implicated in stomach cancer development include high content of nitrates and high salt intake. Accumulating evidence has implicated the role of *Helicobacter pylori* (*H. pylori*) infection in the pathogenesis of gastric cancer. The development of gastric cancer is a complex, multistep process involving multiple genetic and epigenetic alterations of oncogenes, tumor suppressor genes, DNA repair genes, cell cycle regulators, and signaling molecules. A plausible program for gastric cancer prevention involves intake of a balanced diet containing fruits and vegetables, improved sanitation and hygiene, screening and treatment of *H. pylori* infection, and follow-up of precancerous lesions. The fact that diet plays an important role in the etiology of gastric cancer offers scope for nutritional chemoprevention. Animal models have been extensively used to analyze the stepwise evolution of gastric carcinogenesis and to test dietary chemopreventive agents. Development of multitargeted preventive and therapeutic strategies for gastric cancer is a major challenge for the future.

Key words: Chemoprevention; Diet; Epidemiology; Epigenetic changes; Gastric cancer; Genetic alterations; *Helicobacter pylori*; Risk factors

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INTRODUCTION

Adenocarcinoma of the stomach, a leading cause of cancer death worldwide is the second and fourth most common cancer in males and females respectively[1,2]. Globally, gastric cancer accounts for 989 600 new cases and 738 000 deaths annually. The case-fatality ratio of gastric cancer is higher than for common malignancies like colon, breast, and prostate cancers[3]. Despite advances in diagnosis, the disease is usually detected after invasion of the muscularis propria, because most patients experience vague and nonspecific symptoms in the early stages and the classic triad of anemia, weight loss, and refusal of

meat-based foods is seen only in advanced stages. Furthermore, surgery and chemotherapy have limited value in advanced disease and there is a paucity of molecular markers for targeted therapy. Since cancer of the stomach has a very poor prognosis and the 5-year survival rate is only around 20 per cent, a new look at the results of epidemiological and experimental studies is important to establish strategies for primary prevention. This review discusses what is currently known about the pathology, epidemiology, etiology, genetic and epigenetic alterations, and chemoprevention of stomach cancer.

EPIDEMIOLOGY

Age, sex and site distribution

Stomach cancer incidence is known to increase with age with the peak incidence occurring at 60-80 years. Cases in patients younger than 30 years are very rare[4,5]. In India, the age range for stomach cancer is 35-55 years in the South and 45-55 years in the North. The disease shows a male preponderance in almost all countries, with rates two to four times higher among males than females[3,6].

Gastric cancer can develop both in the proximal and the distal region. Distal gastric cancers predominate in developing countries, among blacks, and in the lower socio-economic groups. Dietary factors and *Helicobacter pylori* (*H. pylori*) infection are major risk factors for the development of distal tumors. Proximal tumors are more common in developed countries, among whites, and in higher socio-economic classes. The major risk factors for proximal cancers are gastroesophageal reflux disease and obesity. Distal tumors continue to predominate in Japan in contrast to the increasing prevalence of proximal tumors in the rest of the world[7].

Geographic distribution

The steady decline in the incidence and mortality of stomach cancer in most affluent countries has been attributed to changes in dietary pattern, food storage, and control of *H. pylori* infection. The incidence of gastric cancer varies in different parts of the world with highest incidence rates documented in Eastern Asia, Eastern Europe, and South America, while North America and Africa show the lowest recorded rates[3,8-10]. Stomach cancer is the fifth most common cancer in Europe with 159 900 new cases and 118 200 deaths reported in 2006[11]. The population of Linxian, China is known to have one of the highest rates of oesophageal/gastric cardia cancer in the world[12]. In India, the incidence of gastric carcinoma is higher in the southern and north-eastern states with Mizoram recording an age-adjusted rate of 50.6 and 23.3 for men and women respectively[13,14]. A recent assessment of 556 400 deaths due to cancer in India in 2010 based on a nationally representative survey found that stomach cancer with a mortality rate of 12.6% is the second most common fatal cancer[15].

Significant variations in the incidence of gastric cancer have been observed between different ethnic groups living in the same region; African-Americans, Hispanics and Native Americans are affected more than Caucasians in the United States. High frequency of gastric cancer has been documented in Maoris of New Zealand[16]. However, the geographical distribution of gastric cancer cannot be ascribed to racial differences alone. For example, natives of Japan and China living in Singapore have higher rates than their counterparts in Hawaii. Furthermore, people who migrate from high incidence areas such as Japan to low-incidence regions such as the United States were found to have reduced gastric cancer risk[9,16].

PATHOLOGY

Stomach cancer refers to any malignant neoplasm that arises from the region extending between the gastroesophageal junction and the pylorus. Approximately 95 per cent of stomach tumours are epithelial in origin and designated as adenocarcinomas. Adenosquamous, squamous, and undifferentiated carcinomas are however rare[17]. The World Health Organization and Lauren's classification system have described two histological types of gastric cancer that are clinically and epidemiologically distinct entities- intestinal and diffuse. The well-differentiated intestinal-type, which contains cohesive neoplastic cells, forms gland-like tubular structures that frequently ulcerate whereas the poorly differentiated diffuse-type is characterized by infiltration and thickening of the stomach wall ("leather bottle appearance") without the formation of a discrete mass. The intestinal-type, more common in men, older people in high-risk regions, and in African-Americans, is of the epidemic type and has a better prognosis. It arises from precancerous lesions such as gastric atrophy and intestinal metaplasia, and is influenced by environmental factors such as *H. pylori* infection, obesity, and dietary factors. The diffuse-type represents the major histological type in endemic areas, is more frequent in women and younger patients, and is associated with blood group A, indicating genetic susceptibility. Mixed gastric carcinomas composed of intestinal and diffuse components have also been identified[18,19].

The development of invasive gastric carcinoma involves a stepwise evolution through a cascade of precancerous lesions. Sequential histopathological changes take place in the gastric mucosa including atrophic gastritis with loss of parietal cell mass, intestinal metaplasia, and dysplasia that eventually lead to carcinoma. The metaplasia/dysplasia/carcinoma sequence is more relevant for the intestinal-type gastric cancer that develops by a cumulative series of genetic alterations similar to those in colorectal cancer[20].

ETIOLOGY

Although the etiology of gastric cancer is multifactorial, more than 80% of cases have been attributed to *H. pylori* infection. In addition, diet, lifestyle, genetic, socioeconomic and other factors contribute to gastric carcinogenesis.

H. pylori

H. pylori, a Gram-negative microaerophilic, spiral bacterium found in the gastric mucosa in patients with severe gastritis and chronic atrophic gastritis, has been recognized as an important risk factor for gastric cancer[2,21]. The results of several meta-analyses concluded that *H. pylori* infection is associated with an approximately two-fold increased risk of developing gastric cancer[22]. In a prospective study involving 1526 Japanese patients who had duodenal ulcers, gastric ulcers, gastric polyps or non-ulcer dyspepsia, 2.9% of *H. pylori* infected patients subsequently developed gastric cancer while none of the uninfected patients developed tumors[23]. In 1994, the International Agency for Research on Cancer categorized *H. pylori* as a "Group 1 human carcinogen" based on a plethora of studies[24].

Currently, approximately 50 per cent of the world' s population is infected by *H. pylori*. The prevalence of *H. pylori* infection varies markedly in different countries in Asia with seroprevalence rates higher in developing countries than in industrialized, developed nations[25].

The identification of *H. pylori* as a risk factor for gastric carcinogenesis has stimulated extensive research on the mechanisms by which *H. pylori* induces carcinogenesis. A combination of a virulent organism, a permissive environment, and a genetically susceptible host is considered essential for *H. pylori*-induced gastric cancer. *H. pylori* has been suggested to trigger a cascade of events that promote the sequential progression of normal gastric epithelium through atrophic gastritis, intestinal metaplasia, and dysplasia to carcinoma[26-28]. The bacterium secretes several products that cause gastric mucosal damage such as urease, protease, phospholipase, ammonia, and acetaldehyde. *H. pylori* disrupts gastric barrier function *via* urease-mediated myosin Ⅱ activation[29].

Generation of oxidative stress is recognized as a virulence factor in *H. pylori*-infected hosts. *H. pylori* infection induces the production of reactive oxygen and nitrogen species and suppresses the host antioxidant defense mechanisms, leading to oxidative DNA damage. However, *H. pylori,* which is endowed with a variety of antioxidant enzymes is spared from oxidative stress and the damage is solely restricted to the gastric mucosa of the susceptible host[30]. *H. pylori* although not directly mutagenic, has been suggested to favor the formation of mutagenic substances through inflammatory mediators or by impairing the mismatch repair pathway[26,31]. Kim *et al*[26] demonstrated that *H. pylori* infection promotes gastric carcinogenesis by increasing endogenous DNA damage whilst decreasing repair activities and by inducing mutations in the mitochondrial and nuclear DNA. Aberrant DNA methylation induced by *H. pylori* infection has been found to be a significant risk factor for gastric cancer[32].

Epidemiological evidence suggests that *H. pylori* strains containing the cag pathogenicity island (cagPAI) are more virulent. The cagPAI is a 40-kb genome segment that encodes approximately 30 genes including the cytotoxin-associated gene A (*cagA*). The virulent *cagA* positive strains increase the risk of non-cardia gastric cancer of both intestinal and diffuse types, but not the risk of cardia cancer. The CagA protein is delivered into gastric epithelial cells where it undergoes tyrosine phosphorylation by SRC family kinases. Phosphorylated CagA specifically binds to and activates SHP2, a phosphatase that transmits positive signals for cell growth and motility. Thus *H. pylori* acting *via cagA* activates growth factor receptors, increases proliferation, inhibits apoptosis, and promotes invasion and angiogenesis[33].

Gene expression profiling of gastric antral mucosa samples from *H. pylori* infected patients by microarray analysis followed by quantitative real-time PCR assays have revealed differential expression of 38 genes, indicating that *H. pylori* infection leads to evasion of host defense, enhanced inflammatory and immune responses, activation of NF-κB and Wnt/β-catenin signaling pathways, perturbation of metal ion homeostasis, and induction of carcinogenesis[34].

Dietary factors

A survey of literature on the role of diet in the pathogenesis of gastric cancer using PubMed as a search platform has revealed over 2000 epidemiological and experimental studies. Populations at high risk for stomach cancer have been shown to consume diets rich in starch and poor in protein quality, and are not inclined to eat fresh fruits and vegetables. Both high starch and low protein diet may favor acid-catalyzed nitrosation in the stomach and cause mechanical damage to the gastric mucosa[35-37]. Using an ecological approach, Park *et al*[38] found a negative association between refrigerator use, fruit intake, and gastric cancer mortality and positive associations between salt/sodium intake and gastric cancer mortality and incidence in Korea.

Both epidemiological and experimental studies strongly support the role of excessive salt intake in gastric carcinogenesis. D'Elia *et al*[39] reported a direct correlation between dietary salt intake and risk of gastric cancer with progressively increasing risk across consumption levels based on a meta-analysis of prospective studies. Consumption of large amount of salted fish, soy sauce, pickled vegetables, cured meat and other salt-preserved foods enhances *H. pylori* colonization, and increases the risk of gastric cancer through direct damage to the gastric mucosa resulting in gastritis. Salt is also known to induce hypergastrinemia and endogenous mutations, promoting epithelial cell proliferation which eventually leads to parietal cell loss and gastric cancer progression[40,41]. Reports from this laboratory as well as by other workers have demonstrated that saturated sodium chloride (S-NaCl) promotes the development of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG)-induced rat gastric carcinomas[42,43].

Dietary nitrates are found either naturally in foods such as cabbage, cauliflower, carrot, celery, radish, beets, and spinach or added during preservation. In addition, the nitrate content of fertilizers, soil, and water also contribute to dietary nitrate. Nitrite, nitrate, and nitrosating

agents can be synthesized endogenously by reactions mediated by bacteria and/or activated macrophages. Nitrosation of a number of naturally occurring guanidines and L-arginine-containing polypeptides produces mutagenic compounds. Dietary nitrate is converted to carcinogenic N-nitroso compounds (NNC) by gastric acid thereby increasing gastric cancer risk. Small quantities of preformed NNC and nitrosamines may also be present in some foods including cured meats, dried milk, instant soups, and coffee dried on direct flame[44-46].

In addition to specific components of the diet, certain cooking practices are also associated with increased risk of gastric cancer. These include broiling of meats, roasting, grilling, baking, and deep frying in open furnaces, sun drying, salting, curing, and pickling, all of which increase the formation of NNC. Polycyclic aromatic hydrocarbons such as benzo[a]pyrene formed in smoked food have been incriminated in many areas of the world with high stomach cancer rates[47,48].

Lifestyle

Alcohol, a gastric irritant is an important risk factor for gastric cancer. Zaridze *et al*[49] have reported an increased risk of stomach cancer in men and women who regularly consume strong alcoholic beverages. A direct correlation was observed between consumption of alcohol and tobacco and the risk of gastric cancer in a population-based prospective cohort study[50]. A study from this laboratory demonstrated a positive correlation between alcohol consumption and cigarette smoking with the blood lipid profile in gastric cancer patients[51]. The European Prospective Investigation into Cancer and Nutrition (EPIC) project found a significant association between the intensity and duration of cigarette smoking and gastric cancer risk[52]. Smoking history was found to be a significant independent risk factor for death from gastric cancer in patients who had undergone curative surgical resection[53]. Smoking is known to decrease prostaglandins that maintain gastric mucosal integrity[54]. Tobacco smoke has been reported to induce the development of precursor gastric lesions such as gastritis, ulceration, and intestinal metaplasia. Smokers tend to have a higher incidence of *H. pylori* infection and gastroduodenal inflammation than non-smokers[55].

Family history

Gastric cancer is a known manifestation of inherited cancer predisposition syndromes similar to hereditary nonpolyposis colon cancer and Li-Fraumeni syndrome. According to the OMIM database, 90 per cent of gastric cancers are sporadic, whereas 10 per cent are hereditary. The first documented report of familial predisposition to gastric cancer was described for Napoleon Bonaparte's family (OMIM_192090) with Napoleon, his father, grandfather, brother, and three sisters, all dying of stomach cancer at a relatively early age[56]. The Scandinavian twin study in the Swedish, Danish, and Finnish twin registries found an increased risk of stomach cancer in the twin of an affected person[57]. Family members usually share the same environment and have similar socioeconomic status. These risk factors act independently or in conjunction with genetic factors thereby increasing the risk of stomach cancer.

Occupations

A positive correlation has been recognized between increased stomach cancer risk and a number of occupations including mining, farming, refining, and fishing as well as in workers processing rubber, timber, and asbestos[58,59]. Occupational exposure to dusty and high temperature environments such as in cooks, wood processing plant operators, food and related products machine operators was associated with a significant increased risk of gastric cancer of the diffuse subtype[60]. A German uranium miner cohort study however found a positive statistically non-significant relationship between stomach cancer mortality and occupational exposure to arsenic dust, fine dust, and absorbed dose from α and low-linear energy transfer radiation[61].

GENETIC AND EPIGENETIC ALTERATIONS IN STOMACH CANCER

The development of gastric cancer is a complex, multistep process involving multiple genetic and epigenetic alterations in oncogenes, tumor suppressor genes, DNA repair genes, cell cycle regulators, and signaling molecules. The catalogue of gene alterations in gastric cancer is expanding rapidly[62,63]. An average of 4.18 genomic alterations has been suggested to be necessary for the development of gastric cancer[64]. Gastric carcinoma is characterized by genomic instability that could be either microsatellite instability (MSI) or chromosomal instability (CIN)[65].

CIN

CIN, recognized as the most common instability occurring in sporadic gastric tumors, may manifest as gain or loss of whole chromosomes (aneuploidy) or parts of chromosomes [loss of heterozygosity (LOH), translocations, and amplifications][65]. Comparative genomic hybridization analysis has revealed numerous DNA copy number variations with gains in chromosomal regions 6p21, 9p34, 11q23, 17p13, 19p13, and 22q13, especially in younger patients[66]. Using laser microdissection, Tsukamoto *et al*[67] demonstrated DNA copy number variations in gastric cancer patients with a high frequency of 20q13 chromosome gain as well as upregulation of 114 candidate genes in the regions of amplification, and downregulation of 11 genes in the regions of deletion. LOH at chromosomes 1p, 2q, 3p, 4p, 5q, 6p, 7p, 7q, 8p, 9p, 11q, 12q, 13q, 14q, 17p, 18q, 21q, and 22q that are possible sites of tumor suppressor genes is believed to play a crucial role in gastric carcinogenesis. A high frequency of LOH was found in the adenomatous polyposis coli (APC), p53, nm23 and Rb loci[65,68]. Several factors have been suggested to contribute to CIN in gastric cancer patients including aberrations in chromosome segregation, DNA damage

response, cell cycle regulation, *H. pylori* infection, tobacco, and dietary nitrates.

MSI

MSI, resulting from errors in DNA replication is seen in 15-20 per cent of gastric cancers with a higher frequency in familial cases. The high frequency of MSI associated with advanced, invasive, intestinal-type gastric cancer has been suggested to be due to epigenetic inactivation of the mismatch repair gene *hMLH1*, whereas mutations in transforming growth factor-β (*TGF*-β) *RII*, insulin-like growth factor Ⅱ (*IGF*Ⅱ) *R*, and *BAX* genes in sporadic gastric tumors with MSI display a decreased tendency for invasion and nodal metastasis[65,69,70]. Cytosine-adenine repeat instability, LOH of the APC, and deleted in colon cancer genes have been documented in well-differentiated tumors[71].

Oncogenes

Mutational activation and/or amplification of several oncogenes has been documented in gastric cancer. The K-ras oncogene was found to be mutated (codon-12) in intestinal-type cancer and its precursor lesions, intestinal metaplasia, and adenoma, but not in diffuse-type cancer[72]. Overexpression of *c-erbB2* a cell surface receptor of the tyrosine kinase family is more common in intestinal-type gastric cancer, whereas in diffuse-type gastric cancers, amplification of c-met, a transmembrane tyrosine kinase receptor, and aberrations in the FGFR2/ErbB3/PI3 kinase pathway have been frequently documented[63,73]. A high correlation was observed between EZH2 the human homolog of the Drosophila protein "Enhancer of Zeste", with intestinal-type cancer and the risk of distant metastasis[74].

Tumor suppressor genes

Alterations in a number of TSGs have been documented in the pathogenesis of stomach cancer. The *p53* gene is frequently inactivated in gastric carcinomas as well as in precursor lesions by LOH, missense mutations, or frameshift deletions[63,65,72]. GC-AT transitions of the *p53* gene are common in diffuse-type gastric cancer induced by carcinogenic N-nitrosamines produced from dietary amines and nitrates[72,75]. LOH and mutations of *PTEN* on chromosome 10q23.31 were observed in gastric cancers as well as in precancerous lesions[76]. The *RUNX3* gene, a tumor suppressor, is also involved in the complex process of gastric oncogenesis[77]. Hypermethylation of *RUNX3* promoter in chronic gastritis, intestinal metaplasia, and gastric adenomas, suggests that this gene is a target for epigenetic gene silencing in stomach cancer[78]. Hypermethylation of nuclear retinoic acid receptor β has been documented in intestinal-type gastric cancers but not in the diffuse-type[79].

Cell cycle regulators, growth factors and cytokines

Gene abnormalities and aberrant expression of cell cycle regulators play a pivotal role in the pathogenesis of gastric cancer. Overexpression of *cyclin E* and *CDK* together with aberrant *p53* expression and downregulation of *p27*, a common event in gastric cancer, is associated with increased aggressiveness and poor prognosis[80,81]. A meta-analysis of cell proliferation-related genetic polymorphisms revealed a significantly higher risk of diffuse-type of stomach cancer in individuals harboring TP5372Pro polymorphisms[82]. Immunohistochemistry and TUNEL staining performed on tissue array slides containing 293 gastric carcinoma specimens showed a positive correlation between the expression of *cyclin D1*, *p21*, or *p27* with early pTNM stages, tumor cell proliferation and good prognosis, but an inverse correlation with lymph node metastasis. However, *p27* expression inversely correlated with the apoptosis index indicating that these cell cycle regulators may serve as candidate molecular markers for early gastric carcinoma[83]. High levels of circulating cell-free human telomerase reverse transcriptase mRNA in gastric cancer patients suggests that this molecule may be useful as a noninvasive diagnostic and prognostic marker[84].

Several growth factors and cytokines produced by the gastric tumor microenvironment regulate differentiation, activation, and survival of multiple cell types. Extensive changes in the expression profiles of the components of the TGF-β signaling pathway and its downstream targets occur during the sequential progression of the normal epithelium through chronic atrophic gastritis and dysplasia to carcinoma. These changes include a progressive increase in the expression of *TGFB1/2*, *TGFBR1*, *MYC* and *TP53*, enhanced expression of *SMAD4*, *CDKN1A*, *SMAD1/2/3*, *SMAD2/3* and *CDKN1B* in dysplasia that decreased in carcinoma, and enhanced expressed of *TGFBR2*, *SMAD7*, *RELA*, and *CDC25A* both in dysplasia and carcinoma[85]. A systematic review and meta-analysis of interleukin (IL)-1B cluster gene polymorphisms at positions -511, -31, and +3954 and the receptor IL-1RN variable number tandem repeat (VNTR) polymorphisms revealed that IL-1B -511 T allele and IL-1 RN*2 VNTR are significantly associated with an increased risk of developing gastric carcinoma especially the non-cardia or intestinal-type and among Caucasians[86].

Invasion and angiogenesis

Mutational inactivation and downregulation of genes encoding cell-adhesion molecules that function as tumor suppressors have been documented in gastric cancer. Inactivation of E-cadherin, a product of the *CDH1* gene has been suggested to play an important role in cell motility, growth, and invasion of gastric cancer[87]. Rare genetic alterations of IQ motif-containing GTPase-activating protein 1 gene, also called *p195* (locus 15q26), a negative regulator of cell-cell adhesion at adherens junctions were found to occur in diffuse gastric cancers[88].

Expression of the proangiogenic vascular endothelial growth factor (VEGF) was demonstrated to correlate with poor survival in gastric cancer patients[89]. VEGF-A was found to be a significant marker for the presence of tumor cells in the bone marrow, whereas VEGF-D is a

useful predictor of the lymphatic spread of tumor cells in gastric cancer patients indicating that the metastatic spread of gastric cancer could be determined, in part, by the profile of VEGF family members expressed in the primary tumour of gastric cancer patients[90]. Using human gastric cancer specimens, *in vitro* cell experiments, and *in vivo* animal experiments, Lee *et al*[91] demonstrated that hypoxia-independent promotion of the AKT-HIF-1α-VEGF pathway contributes to gastric cancer tumorigenesis and angiogenesis.

Microribonucleic acids (miRs)

miRNAs located within regions of LOH, amplification, fragile sites, and in other cancer-associated genomic regions regulate a number of important biological processes relevant to carcinogenesis including proliferation, apoptosis, differentiation, angiogenesis, metastasis, and immune response and they function as both oncogenes and tumor suppressor genes. miR dysregulation plays a key role in the pathogenesis of gastric cancer. Studies have shown that miRs that function as oncogenes, such as *miR-21*, *miR-106a* and *miR-17*, were upregulated, whereas miRs that function as tumor suppressors, including *miR-101*, *miR-181*, *miR-449*, *miR-486*, *let-7a*, were downregulated in gastric cancer[92]. In addition, genetic polymorphism of *miR-196a-2* that interfers with its normal binding with target mRNA such as homeobox gene cluster and annexin A1 was associated with a significantly increased risk of gastric cancer[93]. *H. pylori* infection was demonstrated to induce dysregulation of cancer-associated miRNAs including oncogenic (*miR-106b*) and tumor suppressor (*let-7*) miRNAs with hypermethylation of the tumor suppressor miRNAs *miR-124a-1*, *miR-124a-2* and *miR-124a-3*[94,95].

Gene and protein expression profiling

The advent of genomics, proteomics, and transcriptomics has enabled successful detection of the comprehensive molecular alterations that occur during neoplastic transformation of the gastric mucosa. In Japan, a genome-wide linkage study identified chromosome 2q33-35 as a potential susceptibility locus for proximal gastric cancer[96]. Analysis of the microarray gene-expression data of 54 paired gastric cancer and adjacent noncancerous gastric tissues identified gene signatures of different grades and different stages of gastric cancer. While a 19-gene signature distinguished between high- and low-grade gastric cancers, an expanded 198-gene panel allowed the stratification of cancers into four grades plus control and a 10- and 9-gene signature enabled classification of early- and advanced-stage cancer respectively[97]. Sun *et al*[98] analysed multiple gene expression patterns and their exact roles in gastric carcinogenesis using high-throughput tissue microarray technique. The results showed that while *p53* was useful for distinguishing low-grade dysplasia from high-grade dysplasia, high-level expression of *cyclin E* might be an indicator for malignant transformation of dysplasia. Gene expression profiles by Affymetrix technology and quantitative polymerase chain reaction and *in situ* hybridization on tissue microarrays revealed that the majority of alterations associated with early gastric cancer are retained in advanced gastric cancer, with additional gene expression changes in AGC compatible with a progression model of gastric carcinogenesis. Molecular characterization of 8 primary gastric carcinomas, corresponding xenografts, and 2 novel gastric carcinoma cell lines revealed comparable histological features and expression of several markers as revealed by immunohistochemistry, copy number, and hypermethylation of up to 38 genes[99].

Comprehensive protein profiling of paired surgical specimens of primary gastric adenocarcinomas and non-tumor mucosae from Japanese patients by 2-D gel electrophoresis and liquid chromatography-electrospray ionic tandem mass spectrometry revealed increases in manganese dismutase and nonhistone chromosomal protein HMG-1 with decreases in carbonic anhydrases Ⅰ and Ⅱ, glutathione-S-transferase and foveolin precursor (gastrokine-1) (FOV), an 18-kDa stomach-specific protein with putative tumor suppressor activity. RT-PCR analysis also revealed significant downregulation of FOV mRNA expression in tumor tissues, underscoring its potential use as an effective biomarker for diagnosis and molecular target for chemotherapy[100].

Epigenetic changes

Although the role of genetic alterations in gastric cancer has long been recognized, global changes in the epigenetic landscape with reference to DNA methylation, histone methylation and histone acetylation have only been recently documented. While global hypomethylation leads to activation of oncogenes and genomic instability, promoter hypermethylation is associated with transcriptional silencing of TSGs and DNA mismatch repair genes[101]. Diverse CpG island methylator phenotypes have been identified in gastric cancer that serve as good prognostic indicators[102]. Meta-analysis indicated aberrant methylation of 77 genes in gastric cancer, suggesting the potential clinical value of DNA methylation as a marker for risk prediction and prognosis[103]. Hypermethylation of promoters of genes involved in cell cycle control, metabolism of essential nutrients, and production of inflammatory mediators, has been described in *H. pylori* infection as well as in gastric cancer[104].

E-cadherin, a member of the APC pathway, and CDH4 (encoding R-cadherin), are hypermethylated in gastric tumors. In particular CDH4 methylation is an early diagnostic marker for gastrointestinal tumorigenesis[105]. Epigenetic inactivation by hypermethylation of the RAS-related gene, RASSF1A isoform, a negative effector of K-ras, and activation of the R-RAS oncogene by hypomethylation has been reported in gastric carcinomas[106].

Histone acetylation and deacetylation catalyzed by histone acetyltransferases and histone deacetylases (HDACs)

play an important role in chromatin remodeling. Histone H4 acetylation in both the promoter and coding regions of the *p21WAF1/CIP1* gene in cells expressing dominant-negative *p53* was significantly reduced in gastric cancer cells expressing wild-type *p53*[107]. Epigenetic modifications also play an important role in miRNA deregulation in gastric cancer[95].

Thus, genetic and epigenetic alterations can lead to perturbations in normal cellular homeostasis eventually culminating in neoplastic transformation of the gastric mucosa. In particular, disruption in a number of regulatory pathways, evasion of apoptosis and increased progression through the cell cycle could create a permissive environment for genomic instability, invasiveness, and metastasis.

PREVENTION STRATEGIES

Correa *et al*[108] have suggested a plausible program for gastric cancer prevention that involves screening and treatment of *H. pylori* infection, endoscopic and histologic surveillance of precancerous lesions, improved sanitation and hygiene, restriction of dietary salt, and intake of a balanced diet containing fresh fruits and vegetables rich in antioxidants.

Eradication of *H. pylori* infection is regarded as a primary chemoprevention strategy for reducing the incidence of gastric cancer[109]. American and European guidelines recommend *H. pylori* eradication in all patients with atrophy and/or intestinal metaplasia and in all first-degree relatives of gastric cancer patients in addition to endoscopic and histological surveillance. The Asian Pacific Gastric Cancer Consensus has recommended population-based screening and treatment of *H. pylori* infection in regions with an annual gastric cancer incidence above 20/100 000 to reverse *H. pylori*-induced biochemical, genetic, and epigenetic changes. In several intervention trials, *H. pylori* eradication has prevented the progression of precancerous lesions. Intervention studies in Japan have demonstrated significant prophylactic effects of *H. pylori* eradication on the development of gastric cancer. The value of early eradication therapy in preventing gastric cancer development was also confirmed in animal models[109,110].

Modulation of dietary patterns and changes in cooking practices are believed to significantly reduce gastric cancer risk[108]. Refrigeration of food that obviates the use of salt as a preservative, reduces the possibility of molds overgrowing in food, and renders conversion of nitrates into NNC more difficult in cured and pickled foods[38]. Several studies have demonstrated the protective effect of high intake of raw vegetables and fruits against the risk of gastric cancer. A EPIC study that recruited a total of 521 457 subjects in 23 centers across 10 European countries found a positive association between high intake of dietary antioxidants and reduced risk of gastric cancer[111]. Reanalysis of the beneficial effects of fruit and vegetables in a continuation of the EPIC study involving 477 312 subjects including 683 gastric adenocarcinoma patients with 11 years of follow-up found that intake of fresh fruits and citrus fruits protected against the risk of diffuse and cardia gastric cancer respectively[112]. The EPIC study also reported a positive correlation between consumption of red meat and gastric cancer risk, whereas high plasma vitamin C, some carotenoids, retinol and α-tocopherol, high intake of cereal fibre, and adherence to the Mediterranean diet exhibited inverse association[113,114]. Dietary modification by reducing the intake of salt and salted food, as well as by increasing the intake of fruits and vitamin C is thus considered a practical strategy to prevent gastric cancer[37-40]. Both green and black tea consumption has been reported to be associated with reduced risk of stomach cancer in epidemiological and experimental studies[115,116].

Results from epidemiological and experimental studies point to a major influence of antioxidant nutrients in the prevention of gastric carcinogenesis. Low plasma levels of the antioxidants ascorbic acid and vitamin E have been reported in high-risk regions[113]. Studies from this laboratory have demonstrated that patients with gastric cancer are more susceptible to reactive oxygen species-induced lipid peroxidation as a consequence of insufficient antioxidant potential[117]. In particular, vitamin C is reported to prevent gastric cancer development by inhibiting the conversion of nitrates into NNC and to delay tumour induction in experimental animals[118]. Ascorbic acid has been demonstrated to attenuate the mutagenic potency of MNNG in *S. typhimurium* and in gastric mucosal cells[119].

Results from intervention trials confirm that subjects at high risk of developing stomach cancer can be protected by supplementation with antioxidants. The finding of a reduction in cancer mortality among those receiving antioxidant supplements in Linxian, China, was the first large intervention study that stimulated basic research in this area[120].

Dietary antioxidants may exert their inhibitory effects on gastric carcinogenesis by any one or a combination of the following mechanisms- preventing metabolic activation of procarcinogens, inactivating carcinogens, enhancing DNA repair mechanisms, decreasing protooncogene expression, activating tumor suppressor genes, inhibiting cell proliferation, angiogenesis and inflammation, inducing differentiation and apoptosis, stimulating immune response, and modulating transcription factors and aberrant signaling pathways[121].

EXPERIMENTAL CHEMOPREVENTION IN ANIMAL MODELS OF STOMACH CARCINOGENESIS

The fact that diet plays an important role in the etiology of gastric cancer offers scope for nutritional chemoprevention. Chemoprevention, a promising approach for

controlling cancer, involves the use of specific natural or synthetic chemical agents to reverse, suppress or prevent premalignancy from progressing to invasive cancer. Many dietary agents, medicinal plants and their constituent phytochemicals have received growing attention as potential chemopreventive agents over the past few years[122]. However, it is essential to test the chemopreventive efficacy of a putative agent in an animal model of gastric carcinogenesis before embarking on clinical trials.

Various chemical carcinogens such as NNCs, nitro compounds, aliphatic/aromatic hydrocarbons and halogenated hydrocarbons have been reported to induce gastric tumours in experimental animals. A review of the National Toxicology Program database and the Carcinogenic Potency Database revealed that at least 26 chemicals induced gastric neoplasms in rodents of which N-methyl-N-nitrosourea (MNU) and MNNG are the most commonly used[123]. Animal models have been extensively used to investigate the mechanisms of gastric carcinogenesis and to test chemopreventive agents[124].

Tatematsu *et al*[125,126] induced glandular stomach tumors in BALB/c and C3H mice using MNU. Oshima and Oshima[127] constructed a series of mouse models to investigate the role of oncogenic pathways in gastric tumorigenesis. While Wnt activation in gastric epithelial cells suppressed differentiation, and induced preneoplastic lesions, induction of the PGE-2 pathway induced development of spasmolytic polypeptide-expressing metaplasia and promoted gastric hamartoma development when bone morphogenetic protein signaling was suppressed. Simultaneous activation of the Wnt and PGE-2 pathways led to dysplastic gastric tumor development.

The Mongolian gerbil model that develops histopathological changes such as gastric atrophy, intestinal metaplasia, dysplasia, and adenocarcinoma emulates the stages seen in human gastric cancer development. Gastric adenocarcinomas were successfully induced in Mongolian gerbils using MNNG and MNU as well as *H. pylori* infection. The dose-dependent promoting effect of salt was also demonstrated in this model. The Mongolian gerbil has emerged as the most relevant animal model for analyzing gastric cancer development and progression as well as for chemoprevention trials[124,128].

Sugimura *et al*[129] in 1967 first demonstrated that high yields of gastric tumours could be induced in Wistar rats using MNNG. MNNG, a model direct-acting alkylating agent produces several hundred-fold greater alkylation than other alkylating agents. MNNG is known to methylate all oxygen and most nitrogen atoms of DNA. The major mutagenic lesion induced by MNNG is O^6-methylguanine that results in G:C to A:T transition mutations by mispairing during DNA replication[130]. MNNG induces both glandular and forestomach carcinomas, depending on the concentration and route of administration. When administered in drinking water, MNNG predominantly induces glandular stomach tumours. In contrast, intragastric intubation of MNNG either in single or multiple doses is reported to produce forestomach tumours[131].

Experimental gastric tumours induced by the administration of MNNG in Wistar rats show a number of similarities to human stomach cancer. The major risk factors associated with human stomach cancer such as ethanol, high salt, low protein, diet and *H. pylori* were also found to promote or enhance MNNG-induced gastric carcinogenesis[132,133]. Overexpression of *HSP27*, *Bcl-2* and *COX-2*, as well as *H-ras* and *p53* gene mutations have been documented in both human and MNNG-induced gastric tumours[134,135]. Chen *et al*[136] have reported upregulation of 11 proteins and downregulation of 2 proteins in MNNG-induced gastric tumour tissue. The identified proteins include cytoskeletal proteins, stress-associated proteins, and proteins involved in signal transduction, cell proliferation, differentiation, and metabolism. Abe *et al*[137] reported that MNNG-induced rat stomach carcinomas possessed infiltration capacity and had lost differentiated phenotypes for the stomach, in the same way as human stomach carcinomas, and could be used as a good model from the viewpoint of molecular expression profile. A number of dietary agents have been tested for chemopreventive efficacy in the MNNG model, some of which are listed in Table 1.

Studies from this laboratory have demonstrated the inhibitory effects on the development of MNNG-induced rat forestomach tumours of extracts of black tea polyphenols as well as the dietary phytochemicals S-allylcysteine, an organosulfur constituent of garlic, lycopene, a tomato carotenoid, and eugenol, a phenolic constituent found in clove oil[42,116,138-140]. In addition, curcumin, epigallocatechin gallate, folic acid, genistein and naringenin have been reported to exert chemopreventive effects in the MNNG model. Several of these agents act through multiple mechanisms to exert their chemopreventive effects. These include inhibition of genotoxicity and oxidative stress, modulation of signal transduction pathways and genes involved in the control of cell proliferation, cell cycle, apoptosis, invasion, angiogenesis, and transcription regulation[141-145].

MOLECULAR TARGETS FOR CHEMOPREVENTION AND THERAPY

Novel molecular targets are being discovered and used to design drugs for gastric cancer. Most of the strategies for testing the efficacy of gene therapy for gastric cancer have involved the use of adenoviral vectors. Some of the adenovirus-mediated approaches include transfer of *p53*, Bax, truncated dominant negative IGF- I receptor, enhancement of the c-Jun NH_2-terminal kinase to reduce the level of P-glycoprotein, transduction of soluble VEGF receptors Flt-1 in peritoneal mesothelial cells to inhibit the dissemination of gastric cancer *in vivo* and to increase the survival of treated animals[146-150].

Table 1 Dietary agents demonstrated to possess chemopreventive potential in the N-methyl-N'-nitro-N-nitrosoguanidine-induced gastric carcinogenesis model

Agent	Mechanism of action	Targets	Ref.
Curcumin	Inhibition of cell proliferation, angiogenesis and COX-2 signaling	VEGF, COX-2, PCNA	[141]
Eugenol	Inhibition of NF-κB signaling, induction of apoptosis, inhibition of cell proliferation and angiogenesis	NF-κB, IκB, Bcl-2, Bcl-xL, Bax, Apaf-1, cytochrome C, caspase-9, caspase-3, MMP-2, MMP-9, RECK, TIMP-2, VEGF, VEGFR1	[139,140]
Folic acid	Inhibition of cell proliferation	PCNA	[142]
Genistein	Inhibition of cell proliferation and induction of apoptosis	PCNA, Bcl-2, Bax	[143]
Lycopene	Modulation of biotransformation enzymes and antioxidant defenses, induction of apoptosis	Glutathione redox cycle antioxidants, Bcl-2, Bax, Bim, caspase-8, caspase-3	[42,138]
Naringenin	Modulation of biotransformation enzymes and antioxidant defenses	Glutathione redox cycle antioxidants	[144]
S-allylcysteine	Modulation of biotransformation enzymes and antioxidant defenses, induction of apoptosis Induction of apoptosis	Glutathione redox cycle antioxidants, Bcl-2, Bax, Bim, caspase-8, caspase-3	[42,138]
Tea polyphenols and EGCG	Modulation of antioxidant defenses, inhibition of oxidative DNA damage, cell proliferation and angiogenesis, and induction of apoptosis	PCNA, GST-pi, VEGF, Bcl-2, Bax, cytochrome C, caspase-3	[116,145]

MNNG: N-methyl-N'-nitro-N-nitrosoguanidine; VEGF: Vascular endothelial growth factor; VEGFR: VEGF receptors; COX-2: Cyclooxygenase-2; PCNA: Proliferating cell nuclear antigen; NF-κB: Nuclear factor κB; MMP: Matrix metalloproteinase; TIMP: Tissue inhibitor of matrix metalloproteinase; EGCG: Epigallocatechin-3-gallate.

DNA methylating and histone deacetylating markers have assumed significance in recent years for risk assessment, detection, prognostic evaluation, and as therapeutic targets. In particular, the use of HDAC inhibitors that can reactivate transcriptionally silenced genes to induce cell differentiation, apoptosis, and growth suppression is an innovative approach in the treatment of gastric cancer[151]. Nishigaki *et al*[106] demonstrated apoptosis induction in gastric tumor cells by a combination of the deacetylating agent trichostatin A and demethylating agents. However, the clinical efficacy of these agents needs careful evaluation in terms of specific tumor targeting and avoidance of toxic side effects.

CONCLUSION

Stomach cancer is a disease of complex etiology involving multiple risk factors and multiple genetic and epigenetic alterations. Control of *H. pylori* infection by means of eradication or immunization is likely to have immense potential in stomach cancer prevention. In addition, changes in dietary habits and lifestyle could reduce the incidence of stomach cancer especially in high prevalence areas. There is now evidence that mutations in a number of genes as well as genetic polymorphisms are associated with an increased risk for stomach cancer. Despite the availability of new drugs and association regimens, the therapeutic outcome for gastric cancer is still dismal. Knowledge of the diverse risk factors together with current genomic and proteomic technologies would help in identification of high-risk individuals, targeting precursor lesions, improving preventive strategies, and providing appropriate personalized therapy. More rigorous, larger scale and controlled studies are however required to validate the genetic markers. Pharmacogenetics may be an attractive approach to optimize therapeutic regimens and minimize adverse side effects. Multitargeted preventive and therapeutic strategies for gastric cancer are a major challenge for the future.

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