*Current view*

# Gastric Cancer: recent developments in its etiology and pathogenesis

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## SUMMARY

**During recent years significant progress has been made concerning our understanding the role of various factors involved in the etiology and pathogenesis of gastric carcinoma. Several factors are suspected to play a role in gastric carcinogenesis including environmental factors (diet, exogenous chemicals), intragastric synthesis of carcinogens, infectious factors *(Helicobacter pylori)* and genetic ones. Gastric tumorinogenesis is a multifactorial and multistep process, involving accumulation of genetic and epigenetic alterations in oncogenes, tumor-suppressor genes, cell-adhesion molecules, telomere and telomerase activity, as well as genetic instability at several microsatellite loci. These sequential alterations differ between the two histological types of gastric cancer (diffuse and intestinal). Genetic instability, chromosomal instability and immortality participate in the initial step of gastric carcinogenesis. According to the World Health Organization, *Helicobacter pylori* could be classified as a carcinogen of type I. It causes chronic gastritis leading to atrophy, intestinal metaplasia, dysplasia and adenocarcinoma. The knowledge of these events, some of which appear in the early stage of gastric carcinogenesis, could be of value in relation to the prevention strategies and early diagnosis of this lethal condition.**


**Key words:** Gastric cancer, Carcinogenesis, Oncogenes, *Helicobacter pylori,* etiology

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## INTRODUCTION

Despite the decline in the incidence of gastric cancer in many parts of the world and the improvement achieved in the expected survival of operated patients, it remains one of the most frequent and lethal malignancies. It is estimated that there were over 628,000 deaths due to gastric cancer, worldwide in 1990. Gastric cancer is not uniformly distributed among populations, showing marked variation in both incidence and mortality. Several factors are suspected to play a role in gastric carcinogenesis including diet, exogenous chemicals, intragastric synthesis of carcinogens, genetic factors, infectious agents such as infection by *Helicobacter pylori (Hp)* and abnormal gastric conditions, such as gastritis.

During recent years significant progress in our understanding has been made concerning the importance of the various factors related to its etiology and pathogenesis. This review focuses on the recent developments and controversies related to the etiology and pathogenesis of gastric cancer, based on the data of the current literature.

## MOLECULAR BIOLOGY

It is generally accepted that gastric tumorigenesis is a multifactorial and multistep process involving accumulation of genetic and epigenetic alterations in oncogenes, tumor-suppressor genes, cell-adhesion molecules, telomere and telomerase activity, as well as genetic instability at several microsatellite loci. These sequential alterations differ between the two histological types of gastric cancer (intestinal and diffuse type) even though both types may arise from epithelial "stem cells" which express human telomerase reverse transcriptase (hTERT) protein and telomerase activity[1]. The investigation of molecular and genetic changes has brought new insights into the

pathogenesis of gastric cancer. However, there are only a limited number of molecular markers available for gastric cancer detection and prognostic evaluation. The most clinically important molecules whose expression or structure is altered in gastric cancer are shown in table 1[2].

### *Telomerase changes*

Telomerase is an enzyme related to cellular immortality and malignant transformation. It is stringently repressed in most normal somatic cells, although it is reactivated in malignant cells and immortal cell lines, indicating that activation of telomerase may play an important role in carcinogenesis and immortalization. It has been proposed that up-regulation of telomerase expression is associated with gastric cancer development and that up-regulation of telomerase expression detected by *in situ* hybridization could be a useful marker for the early detection of gastric carcinoma[3].

### *Tyrosine kinases*

There is now convincing evidence that tyrosine kinases are involved in gastric carcinogenesis and disease progression. Amplifications of certain tyrosine kinases (c-met, k-sam, and erbB2/neu) have been associated with gastric cancer progression. Alternatively spliced transcripts and enhanced protein-expression levels for some of these tyrosine kinases are correlated with clinical outcomes for gastric cancer patients. Recently, two new gastric cancer molecular markers (tie-1 and mkk4) have been identified through the use of comprehensive tyrosine kinase profiles and demonstrated to be effective as clinical prognostic indicators[4].

### *Metallothioneins expression*

Metallothionein is a small, thiol-rich metalloprotein with antioxidant properties, involved in tumor pathophysiology and therapy resistance[5]. Metallothioneins are expressed in gastric cancer patients, in intestinal metaplasia and dysplasia, as well as in first-degree relatives of patients with gastric carcinoma. It seems that overexpression of metallothioneins is an early process of malignant transformation of the gastric mucosa[6]. Carcinomas with a high level of expression of metallothioneins have an increased malignant potential[5].

### *Gastrin*

It is well established that gastrin is expressed by endocrine tumors and adenocarcinomas of the gastroenteropancreatic region and may represent an autocrine tumor growth factor[7]. Plasma levels of alpha amidated gastrin was several times higher than in normal controls[8].

**Table 1.** Molecules whose expression is altered in gastric cancer.

| |
|---|
| Plasminogen activator |
| Plasminogen activator inhibitor type 1 |
| Cyclin E |
| Epidermal growth factor |
| Bcl-2 |
| E-cadherin |
| Beta-Catenin |
| Germ line mutations of E-cadherin |
| Genetic instability |
| Amplification of Growth factor receptor c-erbB2 |
| Amplification of Growth factor receptor K-sam |

(Becker et al 2000)

### *Cadherin*

E-cadherin, an epithelial cell adhesion molecule, also considered a potential invasion/matastasis suppressor, is encoded by the CDH1 gene. E-cadherin plays an important role in the maintenance of cell-cell adhesion and its function is thought to be regulated by its associated cytoplasmic proteins, such as alpha-catenin and beta-catenin. E-cadherin is mutationally inactivated in nearly half of all undifferentiated-scattered (diffuse-type) gastric carcinomas. In addition, the silencing of E-cadherin by CpG methylation within its promoter region has been reported in several gastric carcinomas cell lines. Loss or downregulation of alpha-catenin expression may be an early event in gastric carcinogenesis[9]. It has also been shown that the E-cadherin promoter frequently undergoes hypermethyliation in human gastric cancers, and can occur early in gastric carcinogenesis[10].

The mutated cadherin gene identified in familial gastric cancer has thrown light onto the pathogenesis. This mutation suggests that an autosomal dominant inheritance with incomplete penetrance could exist in patients with familial gastric cancer. In a recently described family, a germline E-cadherin gene mutation was identified in 5 members of the family, namely, the patient with gastric cancer, her brother and three cousins. Despite the fact that endoscopy in the other four relatives was negative, all underwent subtotal gastrectomy. Pathologic examination of the resected specimens showed multiple foci of signet cell carcinomas in all relatives[11].

### *Cyclooxygenase-2*

It is well accepted that cyclooxygenase is expressed in neoplastic, pre-neoplastic, and peri-neoplastic cells by

mutation of oncogenes, tumor promoters, mitogens, cytokines, their receptors and pathogenisc factors such as Hp. It has been shown that cells overexpressing Cox-2 escape apoptosis, have abnormal cell to cell interactions, and acquire invasive phenotypes. Moreover, both *in vitro* and *in vivo* studies suggest that Cox-2 overexpression upregulates angiogenic factors in neoplastic cells and promotes tumor angiogenesis[12]. Cox-1, the other isoenzyme that is expressed in tumor vascular endothelia, participates in tumor angiogenesis induced by Cox-2 overexpressing cells. Another concept is emerging to indicate that prostaglandins suppress host immunity against tumors. Cox therefore seems to be an important perigenetic factor in the development of cancer growth.

Cox-2 protein over-expression may contribute to an early event of gastric cancer development[13].

Successful eradication of Hp infection leads to downregulation of Cox-2 expression in premalignant and malignant gastric lesions. However it cannot reverse intestinal metaplasia[14] and decrease the risk in those patients with existing, intestinal metaplasia[15].

## GENETIC ALTERATIONS

An accumulation of multiple genetic and epigenetic alterations of oncogenes, tumor suppressor genes, and DNA repair genes are involved in the course of multistep conversion of normal epithelial cells to clinical gastric cancer. Genetic instability, chromosomal instability and immortality participate in the initial step of gastric carcinogenesis. Because telomerase reverse transcriptase protein expression precedes the telomerase activities in precancerous lesions, telomerase reverse transcriptase expression may be a prerequisite for telomerase activation. The cyclin E gene is amplified in 15%-20% of gastric cancer. Reduced expression of a cyclin-dependent kinase inhibitor, p27Kip1, is frequently found in gastric cancer associated with high-grade malignancy. E2F-1, an important downstream target of cyclins/cyclin dependent kinases, is overexpressed in about 40% of gastric carcinomas. Loss of heterozygosity of p73, the p53-related new tumor suppressor gene, preferentially occurs in well-differentiated adenocarcinomas of foveolar type, expressing pS2, a gastric-specific trefoil factor, indicating the importance of p73 loss of heterozygosity in the gastric carcinogenesis[16]. The different molecular genetic alterations in intestinal and diffuse types of gastric cancer have further supported the concept that these two pathological types are different disease entities[17].

### *Oncogenes*

Immunohistochemical expressions of p53 and c-erbB-2 oncogenes are significantly associated with some histopathological phenotypes (advanced cancer of intestinal type). Upregulation of oncogene bcl-2 in premalignant lesions and "downregulation" of the gene after malignant change is a common event. Accumulation of p53 protein is first detected in dysplasia, although mutation of the pS3 gene may occur in intestinal metaplasia. It could be argued that C-erbB-2 expression may be used as a marker for identifying more agressive gastric cancer and thus to design further therapy[18]. Moreover, c-erbB-2 oncogene expression has been linked to the metastatic potential in patients with gastric carcinoma[19].

### *c-met protooncogens*

Amplification of protooncogenes associated with their over-expression is one of the carcinogeneic events identified in gastric cancer. In many cases of gastric carcinoma, a proto-oncogene ERBB-2 is co-amplified with CAB1 genes physically linked to ERBB-2 and both genes are over-expressed. The activation of c-met protooncogene through a rearrangement has been detected in gastric carcinoma tissue and precancerous lesions. Trm-met activation may be an early event in gastric carcinogenesis and may be useful for the identification of individuals with an increased risk of developing gastric carcinoma[20].

### *Ras gene*

H-ras 12 codon mutation has been observed in 32% of patients with advanced gastric cancer although in a significantly lower level in patients with metaplasia and dysplasia[21].

### *Microsatellite instability*

Aneuploidy is considered to be one of the cardinal features of malignant tumors. A second pathway that is not characterized by gross aneuploidy but, instead by inactivation of the DNA mismach repair system, leading to a hypermutable state in which simple repetitive DNA sequences are unstable during DNA replication[22]. Microsatellite instability is a common feature of gastric cancer. This genetic alteration underlines the mismach-repair deficiency in the tumor, caused mainly by methylation of the hMLH1 promoter. Tumors with microsatellite instability have been found to inactivate some target genes by permitting an increased frequency of mutations in mononucleotide runs in their code regions. They also have a distinct clinicopathological profile with a relatively good prognosis. Microsatellite instability affects both the

intestinal and diffuse type of gastric cancer and exhibits marked differences in its incidence in different countries[23]. It promises to be a widely used molecular prognostic test for gastric cancer[24].

## ENVIRONMENTAL FACTORS

### *Diet*

Over the past 20 years, a large number of epidemiological studies have been conducted to investigate the role of diet and the risk of developing gastric cancer. Recently, in relation to evidence has accumulated concerning the exact role of fruit vegetable and salt consumption in the development of gastric cancer. A recently published study from the USA supports a modest role for plant foods in reducing the risk of gastric carcinoma in men but not in women[25].

It has been suggested that consumption of soyfoods may be associated with a reduction in risk of various cancers. A recently published meta-analysis of all studies that obtained individual data on intake of soyfoods and presented risk estimates of the association between intake of fermented soyfoods and risk of gastric cancer, yielded an odds ratio/relative risk of 1.26 (95% CI 1.11-1.43) in association with high intake of such foods[26]. In contrast the analysis of 10 studies with data on non-fermented soyfoods found an odds ratio/relative risk of 0.72 (95% CI 0.63-0.82) in association with high intake of these foods[27]. Further analysis revealed that fermented and non-fermented soyfoods could be associated with salt and fruit/vegetable intake, respectively. At present, the role of soyfoods in the etiology of gastric cancer cannot be fully elucidated until the roles of potential confounders are more adequately adjusted for.

In Korea, an increased risk of stomach cancer was noted among people who frequently consume broiled meat and fish, salted side dishes (salted/fermented fish products) and salty stewed foods such as soybean paste thick stew. Frequent consumption of mung bean, pancake, tofu, cabbage, spinach and sesame oil decrease the risk. Pickled vegetables increased the risk, whereas fresh vegetables did not[28].

Incidence rates of gastric cancer in Japan are much higher than in other countries but have shown a large decline in the past 20 years. It is of interest that incidence rates among the Japanese in Hawaii are only one third or less of the indigenous Japanese in Japan. Epidemiologic studies indicate that consumption of salt or salty food is associated with increased risk of gastric cancer. In contrast, consumption of vegetables and fruit is associated with decreased risk. Occurrence of stomach cancer in Japan may be reduced by two thirds or more via dietary changes, as seen in the population of Japanese ancestry in Hawaii[29].

The role of salt in gastric carcinogenesis seems to be quite important. A high-salt diet has been associated with a high risk of atrophic gastritis and is considered a gastric tumor promoter. Excessive NaCl intake enhances *Hp* colonization both in mice and humans and chronic salt intake may exacerbate gastritis by increasing *Hp* colonization. Elevated salt intake may potentiate *Hp*-associated carcinogenesis by inducing proliferation, pit cell hyperplasia and glandular atrophy[30]. It is of interest that dietary salt does not influence gastric mucosa proliferation in the post-surgical stomach[31]. A diet high in meats may increase the risk of distal gastric adenocarcinoma[32].

Ascorbic acid demonstrates a protective effect against gastric carcinogenesis, due to its ability to inactivate oxygen-free radicals as well as its nitrite-scavenging effects. It has recently been shown that the administration of ascorbic acid significantly helps to resolve intestinal metaplasia of the gastric mucosa following *Hp* eradication[33].

So, at least two practical recommendations – to increase fruit and vegetable intake and to reduce consumption of salt – are supported by the available epidemiological evidence[34]. Promising evidence of a favorable effect of certain vitamins, such as vitamin C and E and beta-carotene, and minerals such as selenium, justifies additional investigation[35].

### *Smoking*

During recent years many epidemiological studies dealing with the role of the smoking habit in the etiopathogenesis of gastric cancer revealed that this environmental factor is contributary in the causation of gastric carcinoma. All the cohort studies performed so far have shown a significantly increased risk of gastric cancer of the order of 1.5 to 2.5 for cigarette smokers, although evidence from case-control studies is less stong.

In a meta-analysis of 40 studies dealing with the association of smoking with gastric cancer, a risk of stomach cancer among smokers of 1.5 to 1.6 as compared with non-smokers was revealed. The summary relative risk was higher in men than in women[37]. In a relevant study performed in the USA, current smoking (in a dose-dependent matter) was found to be a significant risk factor for the development of gastric cardia cancer[28]. In Taiwan cigarette smoking plays the most harmful role in the ini-

tial development of gastric cancer[38]. In a study researching the importance of tobacco smoking and *Hp* infection as risk factors for the development of Gastric carcinoma, it was found that smoking increases the risk of gastric cancer at a level of 2.3 compared with non-smoking *Hp* positive persons[39]. The estimated number of gastric cancer cases directly attributed to the smoking habit worldwide exceed the total number of 80.000 (11% of all cases)[37].

The exact mechanism of gastric cancer promotion by smoking is unknown. It seems, however, that the increased C-X-C chemokine mRNA expression seen in smokers might play a role in inducing enhanced inflammatory activity in gastritis and gastric cancer associated with *Hp* infection[40]. Moreover it seems that smoking is an adverse prognostic factor for gastric cancer (in a dose-dependent matter) increasing the probability of recurrence and diminishing the survival of patients with gastric cancer[41]. It is of interest that the risk of gastric cancer in smokers does not differ by sub-site and histologic sub-type[42].

### *Occupational exposures*

A small excess risk of stomach cancer was found in a metanalysis of published data concerning heavy exposure of workers to lead. However this excess risk may be explained - at least in part - by non-occupational factors[43]. In a large study involving 567 cases of gastric cancer in Sweden, it was found that employment in the metal industry and exposure to phenoxyacetic acids were both positively and independently associated with gastric cancer risk. However, the fractions of all gastric cancers attributable to these job-related exposures were small (7%)[44].

## *HELICOBACTER PYLORI* INFECTION

### *Epidemiology of Hp infection and gastric cancer*

*Hp* infection, together with a number of other environmental factors and individual susceptibility determine the final risk for the development of gastric cancer. However, although a strong link between *Hp* infection and development of gastric cancer is generally accepted, some issues remain still unresolved[45]. These issues include a) differences in the incidence of gastric cancer in favor of men, although the prevalence of infection by *Hp* is the same in both sexes, b) although the prevalence of infection by *Hp* in some developing countries is very high, the incidence of gastric cancer is quite low, c) it is unexplained why, although mortality from gastric cancer is approximately 10 in 100,000 in many countries, the percentage of *Hp* infection varies from 4.2% to 83%, d) The incidence of gastric cancer in patients with duodenal ulcer is much lower compared with the incidence of gastric cancer in the general population, although infection by *Hp* in these patients is greater than 90%, e) Less than 20% of all *Hp* infected persons will develop significant clinical consequences in their life, and f) *Hp* strains are highly diverse at a genetic level and are of different virulence[46].

The existing meta-analyses of all published data concerning the relationship between *Hp* infection and gastric carcinoma support the assumption that *Hp* infection increases the risk of gastric cancer by 2 to 6-fold[47-50] (Table 2).

### *Cag A positivity and gastric cancer*

Human studies suggested that infection with *Hp*, especially that expressing CagA-positivity, is *primum movens* in developing gastric cancer, and the upregulation of growth factors, particularly of gastrin and Cox-2, and dysregulation of the Bax/Bcl-2 system seem to contribute to gastric carcinogenesis[8]. It has been reported that the prevalence of CagA-positive strains was about twice as high (70%) in gastric cancer as in sex and age-matched controls.

### *Growth factors and Hp infection*

Epidermal Growth Factor and Transforming Growth Factor-alpha are expressed more frequently in gastric cancer tissue than in normal fundic mucosa. This factor has been shown to be a potent epithelial mitogen and

**Table 2.** Meta-analyses of the risk of gastric cancer in subjects infected by *Helicobacter pylori*

| Author | Year | No of studies included | Estimated Risk (95% CI) | Type of cancer |
|---|---|---|---|---|
| Helicobacter & C.C.G | 2001 | 12 | 5.9 (3.4-10.3) | non-cardia |
| Feldman RA | 2001 | | 4-fold | non-cardia |
| Eslick et al | 1999 | 42 | 2.04 (1.69-2.45) | non-cardia |
| Huang et al | 1998 | 19 | 1.92 (1.32-2.78) | non-cardia |

oncoprotein when sustained over-expression occurs. It has been shown that the epidermal growth factor and its receptor are increased 2-fold in infection with *Hp* and that eradication of the infection reduces levels of both factors to those of normal controls. It seems that one major pathogenic mechanism for gastric mucosa hyperproliferation and possibly carcinogenesis related to *Hp* infection may be the over-expression of epidermal growth factor and increased receptor density on gastric mucosal cells[51].

### *Eradication of Hp and prevention of gastric cancer*

Eradication of the infection by *Hp* has been shown to prevent the occurrence of metachronous gastric cancer following endoscopic resection for early gastric cancer in Japan. However, the generalization of this conclusion to other populations is difficult because of the vast differences in the definition of gastric atrophy between Japanese and Western pathologists[52].

### *Apoptosis and Hp infection*

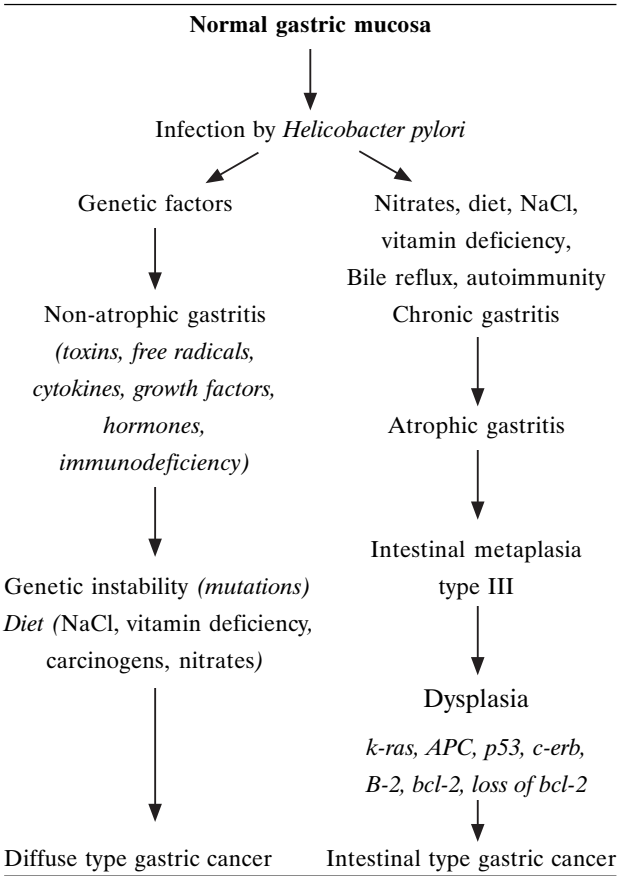
Apoptosis, programmed cell death for superficial aspect of the mucosa, is important for healthy cell turnover. *Hp* infection promotes such a cell death sequence. Because apoptosis regulates the cycle of cell turnover in balance with proliferation, dysregulation of apoptosis or proliferation evoked by *Hp* colonization would be linked to the gastric carcinogenesis. It is generally accepted that under normal situations, apoptotic cells are rare in the glandular neck region (the generative cell zone). With progression of atrophic gastritis, the regerative cell zone shifts downward and a relative large number of apoptotic cells and proliferative cells are present in deeper portions of the glands. A higher frequency of apoptosis has been observed in gastric dysplasia than in coexisting gastric carcinomas, whereas the number of proliferative cells is significantly higher in gastric carcinoma than in dysplasia. *Hp* infection induces apoptosis in gastric epithelial cells, which returns to normal after successful eradication of the infection[53].

Numerous molecules produced by *Hp* including VacA, lipopolysacharide, monochloramine, and nitric oxide may directly induce apoptosis. Moreover, *Hp*-stimulated host inflammatory/immune responses lead to release of a large amount of cytokines. Many of them, including TNF-alpha and Interferon-gamma, markedly potentiate apoptosis. Gastric cell proliferation is significantly higher in patients with *Hp* infection than in normal controls and eradication of the infection leads to a reduction in cell proliferation. It seems that *Hp*-induced apoptosis may play a key role in gastric carcinogenesis by increasing cell proliferation and resulting in gastric atrophy.

### *Chronic gastritis, Intestinal petaplasia and Hp infection*

Infection by *Hp* causes chronic gastritis. In the majority of cases gastritis is localized to the antrum. Chronic inflammation in the antrum results in a reduction in the secretion of somatostatin, increased production of gastrin and consequently increased production of HCl. Patients in whom infection by *Hp* was aquired at an early age usually develop pangastritis leading to gastric atrophy and hypochlorydria. In those patients who will finally develop gastric cancer, atrophic gastritis progresses to intestinal metaplasia, dysplasia and gastric adenocarcinoma. It is of interest that diffuse type gastric cancer, although strongly realted to genetic factors, is also related to *Hp* infection[54]. However there are data not supporting the existence of a relationship between infection by *Hp* and the development of intestinal metaplasia[55].

**Table 3.** A model of gastric carcinogenesis based on the currently available information.



It is supported that the degree of gastritis in first-degree relatives of patients with gastric cancer is more prominent as compared with the degree of gastritis of subjects without a positive family history of gastric cancer. This fact underlines the influence of genetic factors in the degree of severity of gastritis[56].

Prolonged gastric atrophy finally leads to the disappearance of *Hp* infection and reduction of the serum levels of specific antibodies against *Hp*. As the degree of gastric atrophy and intestinal metaplasia increases, the degree of colonization by *Hp* is diminished due to hypochlorydria and bacterial overgrowth in the gastric lumen due to hypochlorydria.

Bearing in mind that atrophic gastritis and intestinal metaplasia are the very initial steps of gastric carcinogenesis it seems that *Hp* infection plays a role in the initial steps of gastric carcinogenesis by inducing chronic inflammation.

Based on the above mentioned data a model of gastric carcinogenesis is shown in table 3.

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