Review article

Molecular basis of gastric cancer development and progression

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Abstract

Gastric cancer is one of the leading causes of cancer death worldwide. Because of its heterogeneity, gastric cancer has been an interesting model for studying carcinogenesis and tumorigenesis. Various genetic and molecular alterations are found in gastric cancer that underlie the malignant transformation of gastric mucosa during the multistep process of gastric cancer pathogenesis. Although the detailed mechanisms of gastric cancer development remain uncertain, the enhancement in understanding of its molecular biology in recent years has led to a better perspective on the molecular epidemiology, carcinogenesis, and pathogenesis of gastric cancer. More importantly, it is becoming possible to use molecular markers in differential diagnosis, prognostic evaluation, and specific clinical interventions. Because multiple molecular alterations are frequently noted in gastric cancer and because its histology is complex, new technologies for studying its molecular biology are important to further evaluate gastric carcinogenesis. This review describes our current knowledge of the molecular basis of gastric cancer as it relates to molecular epidemiology, multiple molecular alterations in pathogenesis, and molecular determinants of invasion and metastasis, as well as their potential clinical applications.

Key words Oncogene · Tumor suppressor gene · Molecular biology · Metastasis · Stomach cancer

Introduction

Although the overall incidence of gastric cancer is decreasing in almost every country, it is still a serious health problem and remains the second most common type of fatal cancer worldwide [1]. Despite the performance of extensive diagnostic and therapeutic investigations of gastric cancer, the prognosis for patients with

advanced gastric cancer remains dismal, and little improvement in survival has been achieved in recent years [2]. One reason is that gastric cancer is still too often diagnosed at an advanced stage, despite the recent studies and efforts on the identification and management of premalignant lesions [3–5]. Over the past several years, there have been many new developments in our understanding of the molecular biology of gastric carcinoma. Many studies have clearly demonstrated that multiple genetic alterations are responsible for the development and progression of gastric cancer [6–9]. It is now evident that different genetic pathways lead to diffuse- and intestinal-type gastric cancer. Alterations in specific genes that play important roles in diverse cellular functions, such as cell adhesion, signal transduction, cell differentiation, development, metastasis, DNA repair, and glycosylation changes, have been identified, although much less is known about the mixed types or rare variants of gastric carcinoma [2–9]. Molecular biology is equally important for developing diagnostic and prognostic molecular markers. Therefore, the challenge is to detect stage-specific genetic abnormalities that may result in early diagnosis and even aid in selecting therapy. This review focuses on the molecular epidemiology and pathogenesis of the major types of gastric cancer. In addition, the molecular diagnosis of and prognosis and therapeutics for gastric cancer also are discussed.

Molecular epidemiology of gastric cancer

Etiology

In recent years, research on gastric cancer has concentrated primarily on identifying environmental and genetic risk factors. Dramatic variations in the incidence of gastric cancer in different geographical 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 [10]. Also, *Helicobacter pylori* infection is considered a risk factor for gastric cancer that may act through different mechanisms, including induction of hyperproliferation, interference with antioxidant functions, and increasing the number of reactive oxygen species and amount of nitric oxide (NO), which may be responsible for oxidative DNA damage [11]. Nevertheless, it has been suggested that *H. pylori* is a cocarcinogen in only a very small proportion of the population infected with the organism [11]. The cofactors involved in the transformation of gastric mucosa to malignancy are unknown, though. Additionally, studies indicate that a high intake of smoked, salted, and nitrated foods and carbohydrates and a low intake of fruits, vegetables, and milk significantly increase the risk of stomach cancer [12]. Other studies suggest that genetic alterations play important roles in the multistage process of gastric carcinogenesis [13]. Hereditary gastric cancer syndromes, which are associated with E-cadherin germline mutations, account for a small minority of patients with gastric cancer [14]. A multifactorial model of human gastric carcinogenesis is currently accepted; different dietary and nondietary factors and genetic susceptibility are involved in this carcinogenesis [13].

Genetic predisposition

There is increasing interest in genetic polymorphisms as causes for gastric cancer, probably due to advances in DNA-analysis technologies [15]. Individual variations in cancer risk have been associated with specific variant alleles of different genes (polymorphisms) that are present in a significant proportion of the normal population. Polymorphisms in a wide variety of genes may modify the effect of environment [13,16]; these gene-environmental interactions may explain the high variation in the incidence of gastric cancer around the world [17]. However, the interaction between environmental factors and genetic susceptibility, at least regarding the metabolic genes, such as *GSTM1*, *GSTT1*, *NAT1*, *NAT2*, and *CYP2E*, has not been adequately addressed [18]. Most of these studies have addressed the effect of genetic variants of metabolic enzymes and inflammation mediators. These variants have since been better characterized, and their association with cancer risk is plausible. The most widely studied polymorphism is the *GSTM1*-null allele. However, only a few studies have evaluated the risk of gastric cancer associated with genes that may cause mucosal protection [15], oxidative damage [19], DNA methylation [20], and DNA repair [21]. The most common result is the increased gastric cancer risk associated with the *IL1B* and *NAT1* variants [15,22–24]. The finding that interleukin-1 (IL-1) gene

cluster haplotypes (*IL1B*-31T and *IL1RN**2/*2), which are thought to increase IL-1 production and inhibition of gastric acid secretion, are associated with an increased risk of both chronic hypochlorhydria and gastric cancer [15] supports the hypothesis that the intensity and maintenance of the inflammatory response to *H. pylori* infection may be relevant to early stages of gastric tumorigenesis [25]. Individual differences in xenobiotic transformation are also important. The observed relationship between fast acetylators (*NAT1**10) and increased gastric cancer risk supports a role in procarcinogen activation in human gastric cancer [23,24].

However, most of the studies of the role of genetic polymorphisms and gastric cancer risk have provided inconsistent results. The disparity in the findings of epidemiological studies may result from limitations in design, the presence of confounding factors, and potential sources of bias that may have significantly affected the conclusions. The limitations of the published literature include potential selection bias for both cases and controls, a limited number of cases without statistical power sufficient to evaluate the gene-environmental interactions [13], mixed cases with different histological types, and anatomic locations that may have different etiologies and genetic predispositions [13].

In summary, major progress has been achieved in the understanding of the etiopathogenesis of sporadic gastric cancer. Clearly, certain environmental and genetic factors contribute to an increased risk of developing gastric cancer. On the other hand, recent studies have also shown that the familial aggregation of gastric cancers relates to numerous factors, including familial clustering of *H. pylori* infection in families, high prevalence of hypochlorhydria and atrophy in infected individuals, other genetic and molecular changes in gastric mucosa that are independent of *H. pylori* infection, and, more importantly, hereditary gastric cancer due to germline mutation of the E-cadherin gene. These discoveries make it possible and important to carry out genetic counseling and testing of asymptomatic family members, and even preventive gastrectomy (for details, see recent review article by Ebert et al. [2]).

Molecular genetics of gastric cancer

Although the pathogenesis of gastric cancer remains largely unclear, the rapid progress in elucidating the molecular biology of cancer has increasingly provided evidence that transformation of a normal epithelial cell to a malignant cell is a multistep process and results from accumulation of multiple gene abnormalities [10,26]. Correa [26] has postulated that a sequence of histomorphological changes leads to gastric cancer.

According to this model, after the development of chronic gastritis, atrophy, intestinal metaplasia, and eventually dysplasia will develop, followed by gastric cancer [26]. This model is still open to debate, and it remains unclear whether these changes follow each other step by step, or whether some histomorphological changes directly precede gastric cancer development [27]. In addition, other authors have interpreted these steps as histomorphological changes that carry an increased risk of gastric cancer [28–31].

A number of molecular abnormalities have been identified, including microsatellite instability (MSI), inactivation of tumor suppressor genes, activation of oncogenes, and reactivation of telomerase [26,27]. Although cancers may harbor multiple molecular alterations, they may not be specific for gastric cancers. Thus, the identified abnormalities may represent only the pathogenesis of gastric cancer, and they have not been identified as a specific sequence of changes leading to gastric carcinoma [6].

MSI

MSI is defined as the presence of replication errors in simple repetitive microsatellite sequences due to DNA mismatch repair deficiency. It is classified as high-frequency (MSI-H), low-frequency (MSI-L), or stable (MSS) [28]. MSI has been recognized as one of the earliest changes in carcinogenesis and results in genomic instability [32]. MSI is present in a subset of gastric cancers [33], ranging from 13% to 44% of gastric tumors [9]; in particular, MSI-H occurs in 10% to 16% of gastric cancers [9]. Furthermore, hypermethylation of CpG islands in the promoter region of the *hMLH1* gene is associated with decreased hMLH1 protein expression and often occurs in gastric cancer cases with MSI-H, indicating that epigenetic inactivation of this gene in association with promoter methylation may underlie MSI [34]. MSI in gastric cancer cases is also associated with antral tumor location, intestinal-type differentiation, and a better prognosis [6,9]. Such cancer cases exhibit a different genetic background from those without MSI, i.e., they exhibit mutations of transforming growth factor (TGF) receptor II and do not have p53 mutations [35]. MSI-H-positive gastric cancers are clinicopathologically distinct; thus, it may be valuable to identify subgroups of gastric cancers [36]. The MSI technique is promising for the early detection of cancer: rather than searching for a specific gene, it may be used as a screening tool [10]. Additional genes with simple, tandem repeat sequences within their coding regions that have been found to be specifically altered in gastric cancers displaying MSI include *BAX*, *hMSH3*, *hMSH6*, *E2F-4*, TGF- β receptor II, and insulin-like growth factor receptor II, which are known to be involved in

the regulation of cell-cycle progression and apoptotic signaling [8,9].

Inactivation of tumor suppressor genes

Inactivation of tumor suppressor genes due to mutations and/or loss of heterozygosity (LOH) is also a frequent event in gastric carcinogenesis. For example, inactivation of p53 and p16 has been reported in both diffuse- and intestinal-type gastric cancers, whereas adenomatous polyposis coli (*APC*) gene mutations seem to occur more frequently in intestinal-type gastric cancers [37–39]. Other candidate tumor suppressor genes that have exhibited genomic alterations in gastric cancers include fragile histidine triad (*FHIT*) and deleted in colon cancer (*DCC*).

Mutation or LOH of the *p53* gene has been reported in up to 80% of gastric cancer cases independent of the histological subtype, and predominately in cases of metastatic disease [39,40–42]. However, *p53* mutations have also been identified in early dysplastic and metaplastic lesions (about 10%) [37,43,44], and some studies have reported that *p53* alterations have a crucial role in early intestinal-type gastric carcinogenesis, likely acting at the transition step between metaplasia and dysplasia, and that the alterations are mainly associated with tumor progression in diffuse-type cancer [37]. However, because of the use of different techniques, including immunohistochemistry, polymerase chain reaction single-strand conformation polymorphism, and sequencing, the results are not consistent [45].

Up to 60% of intestinal-type gastric tumors and approximately 25% of adenomas have mutation and/or LOH of the tumor suppressor gene *APC* [46–50]. These alterations are rare in diffuse-type carcinomas but may be associated with signet-ring cell carcinomas [51]. However, a recent study indicated that somatic mutation of the *APC* gene plays an important role in the pathogenesis of gastric adenoma and dysplasia, but has a limited role in the progression of it to adenocarcinoma [52]. In addition, the APC protein is important for the degradation of β -catenin [53,54]. Specifically, it binds to β -catenin, whose free concentration within the cell is tightly regulated and kept at a low level. After interaction with the transcription factor lymphoid enhancer factor-1 (LEF-1), β -catenin translocates into the nucleus, where it regulates gene expression [55]. β -Catenin mutations have also been detected in intestinal-type gastric tumors, but are absent from diffuse-type tumors [56,57]. In intestinal-type gastric cancers, the accumulation of β -catenin protein may result from impaired degradation of the β -catenin protein due to alterations of the β -catenin and *APC* genes [6]. Both APC and β -catenin are members of the so-called Wnt/wingless signal transduction pathway, which is altered in

more than 90% of colon cancer cases and is currently being intensively analyzed to find small molecules that selectively interfere with its activation in tumor cells.

In previous studies, P16^{INK4} somatic mutations with LOH were noted in several cases of esophageal cancer, but not in those of gastric carcinoma [58,59]. In addition, loss of p16 expression was found to lead to abnormal hypermethylation of the p16^{INK4} promoter, suggesting that this epigenetic gene expression silencing may play a role in esophageal tumorigenesis [60]. On the other hand, a significant portion (>40%) of gastric cancers exhibited CpG island methylation of the promoter region of p16 [61,62]. Many of these cases exhibiting hypermethylation of promoter regions displayed the MSI-H phenotype and multiple sites of methylation, including the hMLH1 promoter region [63,64], suggesting that CpG island hypermethylation occurs early in multistep gastric carcinogenesis.

Aberrant methylation of promoters may lead to the transcriptional silencing of various genes (e.g., E-cadherin, *p16*, *p15*, *MGMT*, and *hMLH1*) in gastric cancer [65–67]. It has been reported that approximately 40% of gastric cancers are CpG island methylator phenotype (CIMP) tumors, which frequently exhibit methylation of the *p16* and *hMLH1* genes [68]. The genetic and molecular changes in these cancers are different from those in CIMP-negative cancers, suggesting an alternative pathway of gastric cancer pathogenesis. Because these changes can also be found in normal mucosa, they are probably early events in gastric carcinogenesis [68].

FHIT is a putative tumor suppressor gene that was isolated from the common fragile site FRA3B at 3p14.2 and found to have abnormal transcripts, with deleted exons in five of nine gastric cancer cases [69]. In other studies, genomic alterations and abnormal expression of *FHIT* were demonstrated in the majority of gastric carcinoma cases [70,71], suggesting that *FHIT* can play an important role in gastric carcinogenesis [71]. A recent study showed that abnormalities of *FHIT*, which are presumably associated with the unstable nature of FRA3B within the gene, are involved in the carcinogenesis of gastric cancer and that lack of mismatch repair possibly promotes its alteration in a subset of gastric cancer cases [72].

The candidate tumor suppressor gene *DCC* is located on chromosome 18q. LOH is frequently observed at the *DCC* locus in well-differentiated but not poorly differentiated gastric cancer [73–76]. Yoshida et al. [74] observed decreased *DCC* mRNA expression in 52 gastric cancers and reported that this decreased expression was closely associated with liver metastasis. A more recent study also reported that altered expression of *DCC* was detectable in gastric carcinomas, albeit more commonly at advanced stages of tumor progression [77].

Yet another gene, trefoil factor family 1 (*TFF1*), resides on chromosome 21q22, a region that has been noted to be deleted in gastric cancers in LOH studies [78]. *TFF1* is synthesized and secreted by normal stomach mucosa and gastrointestinal cells of injured tissue. The link between mouse *TFF1* inactivation and the fully penetrant antropyloric tumor phenotype prompted the classification of *TFF1* as a gastric tumor suppressor gene [79]. There is increasing evidence that *TFF1* is a stabilizer of the mucous gel overlying the gastrointestinal mucosa, which provides a physical barrier against various noxious agents [78]. Altered expression, deletion, and/or mutations of the *TFF1* gene have been frequently observed in human gastric carcinomas [79]. Also, loss of expression of *TFF1* was observed in about 44% of gastric carcinoma cases [78,80], while loss of the trefoil peptide was described in approximately 50% of gastric carcinoma cases [78,81]. Recently, researchers found evidence of a dual antiproliferative and antiapoptotic role of the *TFF1* gene [79].

Activation of oncogenes

The group of activated oncogenes consists primarily of various growth factors and growth factor receptors. For example, in previous studies, the *c-met* protooncogene, which encodes a tyrosine kinase receptor for the hepatocyte growth factor, was overexpressed in 50% of both diffuse- and intestinal-type gastric cancers, indicating poor prognosis on multivariate analysis [50,82–85]. Tumors having overexpression of *c-met* have tended to display increased invasiveness and be poorly differentiated (including scirrhous tumors). Also, *c-met* is amplified and/or overexpressed but not mutated in several tumors, suggesting that overexpression of normal *c-met*, rather than structural alteration, is responsible for activation of the tyrosine kinase receptor [86,87]. Furthermore, rearrangement of the *tpv* and *c-met* genes has been reported in gastric cancer and identified in a small subset of gastric cancers [88].

The *c-erbB2* (*HER-2/neu*) gene, another protooncogene, is a transmembrane tyrosine kinase receptor. *HER-2/neu* protein overexpression or gene amplification is associated with approximately one-fourth of all gastrointestinal tract malignancies [89]. *HER-2/neu* overexpression also appears to be linked with advanced rather than early disease with limited invasion. The majority of studies of this protein have reported a significant prognostic value of *HER-2/neu* status, and overexpression of *HER-2/neu* has been implicated as a potential marker of poor prognosis in gastric cancers [87,90–92]. Other studies, however, have not found any prognostic value of *HER-2/neu* expression in these cancers [93].

Other oncogenes, such as cyclin E, *c-erbB3*, *K-sam*, *Ras*, and *c-myc*, have also been found to be amplified and overexpressed in gastric carcinomas [94–96]. In particular, *K-sam*, which belongs to the fibroblast growth factor receptor family, is frequently overexpressed in diffuse-type gastric cancers, due to amplification of it [45,97]. Additionally, more enhanced expression of the *Ras* gene was found in intestinal-type gastric cancers than in diffuse-type cancers [98] and in advanced rather than in early gastric cancer [99,100]. Expression of *c-myc* has also been found to correlate with the disease stage, depth of invasion [101], and peritoneal dissemination [102].

Telomerase reactivation

Telomeres cover the ends of chromosomes and are important for maintenance of the integrity of the chromosomes [103]. Telomere length gradually shortens in normal cells in the absence of telomerase activity and the telomere acts as a mitotic clock controlling the number of times each cell divides, leading to replicating senescence [104]. While telomeres are shortened during cell division, this shortening is prevented in stem cells and transformed cells by the reactivation of telomerases, which adds a TTAGGG tandem to the 3' end of the chromosomes [105]; thus, the cells do not undergo physiological senescence and acquire transforming capabilities. In addition, the reduction of telomere length may bring about genetic instability and telomerase reactivation. For example, a shorter than normal telomere length was observed in intestinal metaplasia [106], suggesting that this shortening plays a role in early carcinogenesis. In addition, telomerase is expressed during the early stages of gastric carcinogenesis [104]. Reactivation of telomerases has been reported in several malignancies, including gastric cancers, as well as in intestinal metaplasia [107,108].

Molecular determinants of gastric cancer invasion and metastasis

Currently, gastric cancer is one of the best characterized neoplasms at the genetic level [6–9]. In contrast, the molecular mechanisms linked to the aggressive nature of this disease remain poorly understood. It has been suggested that its aggressive nature is related to the activation of various oncogenes and the inactivation of various tumor suppressor genes, as well as abnormalities in growth factors and their receptors, which affect the downstream signal transduction pathways involved in the control of cell growth and differentiation [9–12]. These perturbations confer a tremendous survival and growth advantage to gastric cancer cells, as manifested

often by the development of invasive and metastatic phenotypes that are resistant to all conventional therapies [109]. Growth and metastasis of human gastric carcinoma is a highly selective process involving multiple tumor-host interactions [46,109]. A stressful tumor microenvironment, such as alteration of growth-factor production, oxidative stress, hypoxia, and acidosis, is the cause as well as the consequence of tumor progression [109].

Alterations of growth factors and cytokines

Chaotic production of various growth factors and cytokines is an important component of the tumor microenvironment. Numerous studies have established that human gastric cancer overexpresses many growth factors and their receptors, including the epidermal growth factor (EGF) family [49,50,97,110–112], vascular endothelial growth factor (VEGF) [50,113–115], fibroblast growth factor (FGF) [116,117], and insulin-like growth factor (IGF) [118–120], and numerous cytokines, such as TGF [49,92,121,122], IL-1 [123,124], and IL-8 [125–127]. The abundance of growth-promoting factors and the disturbance of growth-inhibitory factors lead to evasion of programmed cell death and self-sufficiency in growth signals, and to elevated angiogenesis, tumor growth, and metastasis [46,109].

Some mechanisms have already been identified for the aberrant expression of the above growth factors. For example, increasing evidence suggests that VEGF expression is predominantly regulated by hypoxia, which is a common feature of most solid tumors, including gastric cancer. We have recently demonstrated that VEGF expression can be significantly upregulated by low extracellular pH, or acidosis [128], which occur frequently within the expanding tumor mass and particularly in regions surrounding necrotic areas within tumors [129]. Similarly, we have also found that acidosis activates the *IL-8* gene (data not shown). Both VEGF and IL-8 are key angiogenic molecules in gastric cancer. Detailed molecular biology studies have indicated that upregulation of these genes by acidosis may occur through the transactivation and cooperation of the transcription factors, nuclear factor (NF)- κ B and activating protein (AP-1) [130].

However, in many types of tumors, an elevated level of VEGF production can often be detected in tumor cells located in the extreme periphery of the tumor, where there is no apparent hypoxia and acidosis. These observations are consistent with numerous recent findings indicating that such exogenous factors as hormones, cytokines, and growth factors modulate VEGF expression and then angiogenesis [131]. Also, many tumor cells can constitutively express VEGF *in vitro* without any apparent external stimuli [132], which is

consistent with the recent findings indicating that loss or inactivation of tumor suppressor genes and activation of oncogenes are associated with VEGF overexpression [133]. In fact, VEGF promoter analyses have revealed several potential transcription factor binding sites, such as HIF-1, AP-1, AP-2, Egr-1, Sp1, Stat3, and many others [134], suggesting that multiple signal transduction pathways may be involved in VEGF transcription regulation. For example, differential constitutive Sp1 activation is essential for different VEGF expression [132]. Our recent studies have also found that constitutively activated Stat3 directly contributes to the constitutive VEGF expression in human cancer cells [135]. Without apparent external stimuli, human cancer cells also constitutively expresses IL-8 through constitutively activated NF- κ B and AP-1 [130,136].

Therefore, aberrant expression of multiple metastasis-related proteins such as IL-8 and VEGF may result from alterations of the activities of several transcription factors. Here, we propose two potential pathways. One mechanism relates to the genetic mutations of oncogenes and/or suppressor genes, such as *Ras* and *p53*, resulting in constitutive activation of the transcription factors. This may be especially true in the early stage of gastric cancer growth. In the late stage of gastric cancer development, however, notable “stress factors”, such as hypoxia and acidosis that are often seen in the tumor microenvironment, further upregulate these metastasis-related proteins through overactivating the transcription factors. Therefore, at advanced stages, uncontrolled tumor growth and the consequent development of a stress environment may enhance tumor angiogenesis, growth, and metastasis. Understanding the expression and regulation of these molecules may shed more light on the pathophysiology of gastric cancer, as well as suggest new targets for preventive and therapeutic approaches to metastatic gastric cancer.

Apoptosis and cell-cycle regulators

Apoptosis, or programmed cell death, plays a fundamental role in multicellular organisms [137]. Genes that regulate apoptosis, such as *FAS*, tumor necrosis factor (*TNF*), and *bcl-2*, contribute to the development of cancer. LOH at the *bcl-2* locus, an apoptosis inhibitor localized at the inner mitochondrial membrane, is associated with intestinal-type gastric tumors [29,45,138,139], while expression of an apoptosis receptor antigen recognized by SC-1 antibody is preferentially seen in diffuse-type tumors [139]. Additionally, studies have found a higher level of apoptosis in gastric tumors in early development than in those showing progression to adenocarcinoma [140–143]. However, Koshida et al. [143] reported lower levels of apoptosis in early-stage gastric cancers when compared with advanced-stage cancers.

Recently, a study showed a significantly higher level of apoptosis and cell proliferation in differentiated than in undifferentiated early-stage gastric cancers, indicating that apoptosis and proliferation are in balance to contain development in the early stages of these cancers [144].

Abnormalities in cell-cycle regulators are also involved in the development and progression of gastric cancers through unbridled cell proliferation [67]. Specifically, genetic alterations and abnormal expression of various cyclins and cyclin-dependent kinases (CDKs), as well as CDK inhibitors, play a role in gastric cancer pathogenesis. Amplification of cyclin E has been found in 10% of diffuse-type and 20% of intestinal-type gastric cancers, particularly at advanced stages of the latter [145]. In addition, overexpression of cyclin E tends to be correlated with advanced tumor stage, invasiveness, and histological grade [67,145]. Furthermore, cyclin D1 overexpression has been found in approximately 50% of gastric cancers, but more commonly in the intestinal type than in the diffuse type [146]. On the other hand, decreased expression of the CDK inhibitor P27 kip1 is frequently associated with advanced gastric carcinomas [147] and is significantly correlated with the depth of tumor invasion and presence of lymph node metastasis [67].

Alterations of adhesion molecules

In addition to the activation of oncogenes and inactivation of tumor suppressor genes, alteration of adhesion molecules seems to be critical for the development of gastric cancer. For instance, E-cadherin is a binding partner of β -catenin and plays a crucial role in establishing intercellular adhesion and the structural integrity of epithelial tissues [148,149]. E-cadherin belongs to the cadherin superfamily and is involved in maintenance of the epithelial phenotype. Reduction or loss of E-cadherin expression has been found often in gastric cancers, probably because of hypermethylation of the E-cadherin promoter [7,150]. A more recent study showed that abnormal E-cadherin expression is a possible marker of submucosal invasion in early differentiated-type gastric cancer and that a lack of β -catenin staining can be used as a predictor of lymph node metastasis in both the intestinal and diffuse types of gastric cancer [151]. In addition, dysfunction of the E-cadherin-catenin complex has been shown to occur at an early stage of gastric carcinogenesis [148]. Somatic mutations in E-cadherin have been detected in 50% of diffuse-type gastric cancer cases; in comparison, loss of the remaining allele, resulting in complete inactivation of the protein, was seen in more than 75% of the cases with mutation [152,153]. Somatic E-cadherin mutations are typically in-frame deletions, removing partial or

complete sequences from the mRNA, or point mutations, resulting in single amino-acid changes [45]. Cell culture experiments have demonstrated that E-cadherin mutations result in markedly reduced cell adhesion, altered morphology, and enhanced cellular motility [154]. Thus, these mutations actively contribute to the morphology of the tumors. E-cadherin mutations are absent from intestinal-type tumors, however. Because of its tumor specificity and biological relevance for malignancy, mutant E-cadherin is an excellent marker for diagnosis and a very attractive target for novel therapeutic interventions [45,48,97,115,155–158].

Nitric oxide synthase (NOS)

Nitric oxide (NO) is a potent biological molecule that mediates a diverse array of activities, including vasodilatation, neurotransmission, iron metabolism, and immune defense [159,160]. Recent studies have suggested that tumor-associated NO, which is, presumably, produced by tumor and/or host cells (e.g., macrophages) that infiltrate and surround tumors, has pleiotropic effects on carcinogenesis, tumor growth, and metastasis [161–166]. The outcome apparently depends on the genetic and epigenetic makeup of tumor cells as well as the source and level of NO production, which itself is dictated by the isoforms of NO synthases (NOSs), i.e., NOS I, NOS II, and NOS III [166]. It is generally believed that a low level of NO production, mostly from elevated expression of NOS I and NOS III (constitutive NOS), most likely benefits tumor growth through increasing tumor blood perfusion and angiogenesis [161,166]. However, expression of NOS II (inducible NOS) can have the opposite effects, mainly because of the high NO production and long-lasting half-life of the NOS II enzyme.

NOS II expression and NO production within tumor cells can directly or indirectly influence the fate of the cells themselves [161–166]. Expression of the *NOS II* gene inversely correlates with the metastatic ability of human colon cancer [167] and K-1735 murine melanoma cells [168]. Also, overproduction of endogenous NO is autocytotoxic through the induction of apoptosis [166] and suppresses tumor growth and metastasis [166,169]. Expression of NOS II in tumor stromal cells, e.g., infiltration macrophages and vascular endothelial cells, has also been implicated in tumor progression and may be the major source of tumor-associated NO [159,160,166,170]. Activated macrophages and endothelial cells may produce NO at cytotoxic levels in vitro [159,166,170] and prevent tumor growth and metastasis, presumably by killing tumor cells passing through vascular lumens [166].

Conversely, the role of NOS II in tumor growth is complex and not fully understood. There is a body of

evidence indicating that NOS II may promote tumor growth and metastasis. Specifically, tumor-associated NOS II activity correlates with more advanced human tumors of the breast [171] and central nervous system [172]. In fact, NOS II expression directly correlates with the metastatic potential of UV-2237 murine fibrosarcoma cells [159]. In addition, macrophage-derived NO may promote tumor growth and metastasis through multiple mechanisms, such as regulation of immune response, cell survival, blood flow, and vessel formation [159,160,173]. Consistently, enforced low levels of NOS II expression have been shown to positively influence tumor progression by protecting tumor cells from apoptosis [174] and altering tumor blood supply through changing vascular tone and/or formation [161,175]. NO also stimulates vascular endothelial cell proliferation and migration directly or indirectly by inducing the expression of VEGF [176,177].

However, the expression and potential role of NOS II and NOS III in the pathogenesis of human gastric cancer are mostly unknown. Recent studies showed elevated NOS II and NOS III expression in human gastric cancer specimens, which has been interpreted as inhibiting or promoting tumor progression [178–184]. Clearly, the definitive roles of NO in gastric cancer development and progression remain to be determined.

Cyclooxygenase (COX)-2

COX-2, one of the two key enzymes in the conversion of arachidonic acid to prostanoids, is the target of inhibition by nonsteroidal anti-inflammatory drugs. Increased expression of COX-2 has been associated with inflammatory processes and carcinogenesis [185,186] and has been detected in several common human malignancies, predominantly of the gastrointestinal tract, including colorectal, esophageal, and gastric carcinomas [187]. COX-2 overexpression is common in intestinal-type gastric carcinoma and dysplastic precursor lesions, suggesting a relatively early role for COX-2 expression in gastric carcinogenesis [188,189]. Additionally, COX-2 overexpression is inversely associated with MSI in gastric cancer [190]. COX-2 overexpression in epithelial cells inhibits apoptosis and increases the invasiveness of malignant cells, favoring tumorigenesis and metastasis [191]. Transcriptional repression of COX-2 has been shown to be caused by hypermethylation of the COX-2 CpG island in gastric carcinoma cell lines [192]. In addition, promoter methylation regulates *H. pylori*-stimulated COX-2 expression in gastric epithelial cells [193]. Loss of COX-2 methylation may facilitate COX-2 expression and promote gastric carcinogenesis associated with *H. pylori* infection [194]. In one study, after successful eradication of *H. pylori*, expression of COX-2 was reduced but not eliminated in the epithelium [195].

Table 1. Major molecular markers of intestinal-type and diffuse-type human gastric cancer, and potential clinical applications

Molecules	Alteration	Percentage		Application	References
		Diffuse	Intestinal		
Tumor suppressors					
p53	Mutation, LOH		20–80	DA, TT	41, 42
APC	Mutation, LOH	30	40–60	DA	48–50
DCC	LOH	0	50	DA	75, 76
CDH1	Mutation	50	0	RA, DA, TT	49, 155–157
β-Catenin	Mutation	0	30	DA	45, 97, 115, 158
Oncogenes					
K- <i>ras</i>	Mutation	0	10	DA, TT	50, 112
<i>bcl-2</i>	LOH	0	40	DA	29, 45, 139
<i>c-met</i>	Amplification	40	20	PF	29, 48, 85
K- <i>sam</i>	Amplification	30	0	PF	45, 97
<i>c-cerB2</i>	Amplification	0	20	PF, TT	83, 92
MSI			10–40	DA, PF	9, 10, 36
Growth factor/receptors					
EGFR	Overexpression	25	50	PF, TT	50, 112
EGF	Overexpression	50	40	PF, TT	48, 97
TGF-α	Overexpression	55	60	PF, TT	50, 92
VEGF	Overexpression	10	45	PF, TT	48, 115
Transcription factors					
CDX1	Overexpression	50	40	PF, TT	200
CDX2	Overexpression	60	50	PF, TT	200
Ets1	Overexpression		65	PF, TT	201, 202
NF-κB	Overexpression		25	PF, TT	203
Sp-1	Overexpression	35	20	PF, TT	115

DA, diagnostic application; PF, prognostic factor; TT, therapeutic target; RA, risk assessment; MSI, microsatellite instability; LOH, loss of heterozygosity

Other candidate genes that have been identified to play an important role in cell-cell adhesion and metastasis include *CD44* [196], *nm23* [197], matrix metalloproteinase-2 [10], and plasminogen activator type 1 (*PAI-1*) [198]. Moreover, the expression and regulation of mucin and glycoconjugate also play important roles in the pathological processes of *H. pylori* infection and gastric carcinogenesis [199]. Interestingly, several recent studies have shown that the aberrant expression of transcription factors contributes to gastric cancer development and progression. These factors included CDX1 [200], CDX2 [200], Ets1 [201,202], NF- κ B [203], and Sp1 [115]. For example, Sp1 overexpression has been shown to predict poor clinical outcome of gastric cancer [115]. Major molecular markers for the diffuse- and intestinal-type of gastric cancer and their potential applications are summarized in Table 1.

Molecular changes associated with *H. pylori* infection

The etiological features of *H. pylori* in gastric carcinoma are based mainly on epidemiological studies [1]. Although it is fairly well accepted that *H. pylori* infection plays a role in causing gastric cancer, the exact

mechanisms involved in its pathogenesis are unclear. *H. pylori* infection leads to different clinical and pathological outcomes in humans, including chronic gastritis, peptic ulcer disease, and gastric carcinoma [1]. *H. pylori* also was recently shown to be associated with molecular changes that developed in the gastric carcinogenesis process [204].

Accumulating evidence suggests that *H. pylori* induces cell proliferation and apoptosis during early chronic inflammation of the gastric mucosa, whereas in malignant transformation of the gastric mucosa, apoptosis is inhibited, and adhesion of gastric epithelial cells is impaired [2]. Studies have shown significantly increased apoptotic cells in *H. pylori*-infected gastric tissues throughout the depth of gastric glands compared with that in uninfected gastric tissue samples and after *H. pylori* eradication [205,206]. It has also been reported that incubation of cultured gastric cancer cell lines with *H. pylori* strains leads to increased cell proliferation [207]. Also, downregulation of the E-cadherin protein has been associated with *H. pylori* infection in patients with normal gastric mucosa, gastritis, a gastric ulcer, or a duodenal ulcer [208]. Furthermore, a study of individuals with a family history of gastric cancer revealed an association between reduced α -catenin

expression and *H. pylori* infection of the gastric mucosa [209]. Recently, Shibata et al. [210] reported that CagA(+) *H. pylori* infection, when compared with CagA(−) infection or the absence of *H. pylori* infection, was associated with a higher prevalence of *p53* mutations in gastric adenocarcinoma cases. Similarly, Kubicka et al. [211] found more *p53* mutations in patients with serology positive for *H. pylori* (43%) than in patients with *H. pylori*-negative serology (14%) [210,211]. Wang et al. [212] reported that *H. pylori* infection may increase the expression of ras p21 proteins and induce *p53* suppressor gene mutation early in the gastric carcinogenesis process. However, the expression of various oncogenes (e.g., K-ras, *c-erbB2*) and MSI is independent of *H. pylori* infection [212–215]. Interestingly, Churin and colleagues [216] recently found that *H. pylori* CagA protein targets the c-Met receptor and enhances the motogenic response, suggesting that *H. pylori* might be involved in aggressive gastric cancer.

Mucosal growth-promoting factors such as gastrin, hepatocyte growth factor (HGF), and TGF- α may play an important role in the transformation of normal *H. pylori*-infected gastric mucosal cells into malignant cells [216,217]. Specifically, increased production of gastrin and overexpression of HGF and TGF- α contribute to gastric carcinogenesis through activation of the NF- κ B-dependent cytokines IL-8 and TGF- α [216,217]. Recent molecular studies have also revealed that *H. pylori* injects bacterial proteins into the cytosol of gastric cells via the type IV injection system and regulates intracellular signal transduction in host cells [218], which is a novel means of survival. Further molecular evidence is needed to elucidate *H. pylori* infection as an etiological factor for gastric carcinogenesis.

Clinical applications

Molecular diagnosis

Based on the molecular pathology findings described above, it is possible to improve the quality of histopathological diagnosis by analyzing genetic and epigenetic alterations. Understanding the structure and function of gastric cancer-associated genes is fundamental for establishing methods of gastric cancer diagnosis and quantification of an individual's risk of the disease. More and more molecular diagnostic markers are being identified to aid in the diagnosis of gastric cancer, e.g., *p53*, APC, and CD44 have been used as markers for differential diagnosis; epidermal growth factor receptor (EGFR), c-met, and c-erbB2 have been used as markers for the degree of malignancy; and MSI has been used as a marker for the screening of genetic instability [67].

MSI causes accumulation of genetic alterations and has been recognized as one of the earliest changes in

gastric carcinogenesis. Identifying patients with genetic instabilities may help identify those at risk for carcinomas. MSI is detected in both gastric adenoma and intestinal metaplasia, which are precancerous lesions associated with well-differentiated gastric cancers [219]. A recent report showed that MSI and hypermethylation-associated inactivation of hMLH1 were more prevalent in early gastric cancers than in gastric adenomas [220]. Techniques of detecting MSI are promising for the early diagnosis of tumors before they become microscopically obvious.

Researchers have found that inactivation of the *p53* and APC genes in early gastric cancer can be used for early detection, whereas the amplification and overexpression of c-met, K-sam, c-erbB2, and cyclin E, and the overexpression of growth factors are indicators of high-grade tumors [67]. Recently, an increasing number of technologies, such as laser microdissection, have become available for gastric cancer diagnosis. In addition, the DNA microarray technique is a powerful tool for identifying novel genes that participate in the development and progression of gastric cancer [67].

The ability to identify individuals who have an inherited risk of gastric cancer would allow more intensive clinical surveillance, early detection, and improved diagnosis [221]. Recently, direct proof of a distinct gastric cancer syndrome resulted from the identification of germline E-cadherin mutations cosegregating in three large Maori families in New Zealand [221]. Thus far, many inactivating germline mutations in the E-cadherin gene have been identified in families in whom diffuse-type gastric cancer is frequently diagnosed [222]. Many other genetic and epigenetic alterations in the E-cadherin gene are also evident [223–227]. These findings demonstrate that E-cadherin gene alterations are a common cause of gastric cancer.

Prognosis

The use of molecular markers as tumor and prognostic markers seems to be promising. Evaluating molecular markers in relation to clinical outcome should provide information about the use of these genetic alterations as prognostic markers, which could influence the design of a therapeutic concept for individual patients [10,45]. Although prognostic studies have been inconsistent, one recent study showed that *p53* mutations were significantly correlated with poor survival after potentially curative resection of gastric cancer [210]. Also, Sanz-Ortega et al. [228] reported that *p53* protein expression showed a statistically significant association with overall survival and was correlated with cardia location, nodal involvement, and tumor stage in patients with gastric cancer, while c-erbB2 and c-myc were significantly enhanced in well-differentiated tumors with a worse prog-

nosis [229]. Furthermore, overexpression of c-erbB2 has been found in intestinal tumors and may serve as a prognostic marker for tumor invasion and lymph node metastasis; therefore, it may be linked with poor prognosis [228]. Overexpression of some growth factors, such as EGF, EGF receptor (EGFR), TGF- α , and VEGF, correlates with tumor malignancy and lymph node metastasis and indicates a worse prognosis for gastric cancer patients [45]. In addition, Akama et al. [145] have reported that cyclin E overexpression correlates with staging, invasiveness, and proliferation of gastric cancer and may be a marker for aggressiveness. Reduced expression of the cell-cycle inhibitor p16^{MTSI}, which correlates with the depth of invasion and potential for metastasis, was also reported, although somatic mutations of it are very rare [45]. Additionally, studies of MSI have shown a statistically significant association of it with a lack of lymphatic infiltration within the tumor and a tendency for it to be associated with longer survival [230], although more extensive studies are needed to clarify the value of MSI as an independent prognostic factor. Other reported independent prognostic factors include tumor-associated proteases and urokinase-type plasminogen activator (uPA) and its inhibitor (PAI-1). In particular, elevated uPA and PAI-1 levels were shown to be associated with poor prognosis and shorter survival [231,232].

Therapeutic applications

With the better understanding of the molecular mechanisms of gastric cancer, new therapeutic targets are now available. Agents that specifically block the catalytic activity of protein tyrosine kinases provide an alternative strategy for the development of novel antitumor agents [10]. These include naturally occurring compounds isolated from fungal extracts, and the synthetic tyrosine phosphorylation inhibitors, known as tyrphostins [233]. A therapeutic approach for gastric cancer, using monoclonal antibodies, e.g., SC-1 (isolated from a patient with signet-ring cell carcinoma) and CH401 (an anti-erbB2 mouse-human chimeric monoclonal antibody), also has been reported [234,235]. Specifically, SC-1 was shown to react with a 50-kDa surface molecule expressed by gastric carcinoma cells; it induces apoptosis and inhibits proliferation of gastric carcinoma cells in vitro and can significantly reduce gastric cancer growth in vivo [236]. Also, CH401 was reported to be able to kill gastric cancer cells overexpressing the erbB2 protein in vitro by inducing apoptosis in the cells [237]. In addition, E-cadherin mutation-specific monoclonal antibodies were reported to react with 13% of E-cadherin-positive diffuse-type gastric cancers [238]. The concept of the induction of tumor-specific apoptosis by monoclonal antibodies may produce a novel type of

adjuvant cancer therapy [45]. The antibodies may also be used in a gene therapy approach for the introduction of a costimulator to activate the patient's immune system or to generate specific antibodies that recruit immune cells to tumor cells [239].

The recent development of high-throughput technologies for analyzing thousands of single-nucleotide polymorphisms (SNPs) in drug-handling genes may revolutionize the field of pharmacogenomics [240]. Particularly, polymorphisms in metabolizing genes may have the potential to predict the effectiveness of chemotherapy. In this way, the molecular profile of a tumor may suggest a certain drug combination, and the patient's SNP "makeup" may dictate the schedule [241].

Future directions

Gastric cancer is an interesting model for studying carcinogenesis and tumorigenesis because it represents a heterogeneous condition. Although much has been learned about the molecular basis of gastric cancer, the detailed mechanisms of gastric cancer development remain unclear. Various genetic and molecular alterations that are present in gastric cancer underlie the malignant transformation of the gastric mucosa during the multi-step process of gastric cancer pathogenesis. However, the rapid development of molecular biology technologies such as laser-based microdissection techniques [241,242] and cDNA microarrays [243] makes further molecular analysis of gastric cancer possible. Using cDNA microarray analysis, the expression of hundreds of potential molecular markers can be evaluated in a short period of time [244], in contrast with the current capability to assay only a single or few molecules. For example, Boussioutas and colleagues [245] have recently shown some exciting results on the expression signatures that are characteristic of premalignant gastric mucosa or gastric cancers. The identification of apparent mitochondrial stress in chronic gastritis has important implications for the major molecular effects of *H. pylori* infection on parietal and epithelial cells, and possibly for the initiation of the premalignant process of gastric cancer. Intestinal-type gastric cancer is characterized by a proliferative signature, in contrast to diffuse-type gastric cancer, and this signature suggests that patients with intestinal-type gastric cancer may particularly benefit from antiproliferative chemotherapeutic agents [245]. In addition, because of the multiple molecular alterations, as well as complications in the histopathology of gastric cancer, the significance of these molecular changes in gastric pathogenesis remains to be further elucidated. Illustration and characterization of the critical molecular alterations in gastric cancer may hold great promise for screening, enhancing

classification, diagnosis, prognosis, and most importantly, developing therapeutic targets in the near future.

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