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REVIEW

Alterations of tumor suppressor and tumor-related genes in the development and progression of gastric cancer

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Abstract

The development and progression of gastric cancer involves a number of genetic and epigenetic alterations of tumor suppressor and tumor-related genes. The majority of differentiated carcinomas arise from intestinal metaplastic mucosa and exhibit structurally altered tumor suppressor genes, typified by *p53,* which is inactivated via the classic two-hit mechanism, i.e. loss of heterozygosity (LOH) and mutation of the remaining allele. LOH at certain chromosomal loci accumulates during tumor progression. Approximately 20% of differentiated carcinomas show evidence of mutator pathway tumorigenesis due to *hMLH1* inactivation via hypermethylation of promoter CpG islands, and exhibit high-frequency microsatellite instability. In contrast, undifferentiated carcinomas rarely exhibit structurally altered tumor suppressor genes. For instance, while methylation of *E-cadherin* is often observed in undifferentiated carcinomas, mutation of this gene is generally associated with the progression from differentiated to undifferentiated carcinomas. Hypermethylation of tumor suppressor and tumor-related genes, including *APC, CHFR, DAP-kinase, DCC, E-cadherin, GSTP1, hMLH1, p16, PTEN, RASSF1A, RUNX3,* and *TSLC1,* can be detected in both differentiated and undifferentiated carcinomas at varying frequencies. However, the significance of the hypermethylation varies according to the analyzed genomic region, and hypermethylation of these genes can also be present in non-neoplastic gastric epithelia. Promoter demethylation of specific genes, such as *MAGE* and *synuclein γ*, can occur during the progressive stages of both histological types, and is associated with patient prognosis. Thus, while the molecular pathways of gastric carcinogenesis are dependent on histological background, specific genetic alterations can still be used for risk assessment, diagnosis, and prognosis.

Key words: Gastric cancer; *p53; E-cadherin*; *hMLH1*; Methylation

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INTRODUCTION

Ever since the initial report of frequent mutation of the *p53* tumor suppressor gene in primary gastric cancers[1], a growing number of genetic and epigenetic alterations in tumor suppressor and tumor-related genes have been determined to be involved in gastric carcinogenesis. In addition to *p53* mutations, *epithelial (E)-cadherin* mutations are also frequent and appear to be important in gastric carcinogenesis[2,3], such that germline mutations of *p53* and *E-cadherin* have both been associated with hereditary gastric cancer[4,5]. These genes are frequently inactivated by the combination of mutation and loss of heterozygosity (LOH)[1,3], although *E-cadherin* can also be inactivated by promoter hypermethylation[6]. LOH at other chromosomal loci can accumulate during gastric cancer progression[7]. In contrast, mutations of the DNA mismatch repair genes *hMSH2* and *hMLH1* are rare, despite the finding of high-frequency microsatellite instability (MSI) in gastric cancers[8-10]. Recently, hypermethylation of the *hMLH1* promoter CpG island was found to be responsible for the development of the majority of gastric cancers exhibiting MSI[11]. Thus, inactivation of *hMLH1* via promoter methylation leads to MSI, and subsequently to mutations in simple repetitive sequences contained within a number of target genes associated with cell proliferation, apoptosis, or mismatch repair, e.g., *transforming growth factor-β type II receptor* (*TGF-β RII*), *bcl-2-associated X* (*BAX*), *hMSH3*, and *E2F-4*[12]. In addition to *hMLH1*, promoter hypermethylation of tumor suppressor and tumor-related genes such as *APC*, *CHFR*, *COX2*, *DAP-kinase*, *DCC*, *E-cadherin*, *GSTP1*, *HRK*, *LOX*, *MGMT*, *p14*, *p15*, *p16*, *PTEN*, *RASSF1A*, *RUNX3*, *14-3-3 sigma*, *THBS1*, *TIMP-3*, and *TSLC1* has also been described in gastric cancer[6,11,13-36]. Hypermethylation can occur in both neoplastic and non-neoplastic gastric epithelia, and therefore is regarded as an early event in gastric carcinogenesis. Another epigenetic alteration, hypomethylation, also appears to be involved in gastric carcinogenesis. Global DNA hypomethylation is thought

to occur during the early stages of tumor development in gastric and other tissues, similar to promoter hypermethylation described above[37-40]. However, demethylation of individual genes, such as *MAGE* and *synuclein-γ*, probably occurs during the progressive stages of gastric carcinogenesis, after global DNA hypomethylation[41,42].

From a histopathologic point of view, gastric cancers are classified as either differentiated carcinomas, which form tubular or papillary structures (roughly corresponding to the intestinal type), or undifferentiated carcinomas in which such structures are inconspicuous (roughly corresponding to the diffuse type)[43,44]. It was thought that differentiated carcinomas, with a predominantly intestinal cellular phenotype, originated from gastric epithelial cells that had undergone intestinal metaplasia, while undifferentiated carcinomas rose from native gastric epithelial cells[43-45]. However, recent advances in mucin histochemistry and immunohistochemistry indicate that some differentiated carcinomas have a predominantly (and, on occasion, exclusively) gastric cellular phenotype and appear to be derived from foveolar epithelial cells[46,47]. It also appears that gastric cancers can undergo changes in cellular phenotype over time, from gastric to intestinal[48]. Thus, differentiated carcinomas may develop from native gastric mucosa or intestinal metaplastic mucosa. Therefore, although it has been proposed that different genetic pathways exist for differentiated and undifferentiated histological types[49], the tumor types must share some common genetic alterations as a significant proportion of differentiated carcinomas progress to become undifferentiated carcinomas[50]. Indeed, recent studies have indicated that tumor cell phenotype is a marker of particular genetic aberrations[46,47].

In this article, genetic and epigenetic alterations involved in the development and progression of gastric cancer are reviewed in relation to tumor histogenesis.

GENETIC AND EPIGENETIC ALTERATIONS IN GASTRIC CANCER

p53

The *p53* gene product functions as a cellular gatekeeper and plays important roles in cell growth and division. It assists DNA repair by effecting G_1 arrest in the presence of DNA damage, induces DNA repair genes, and initiates apoptosis if DNA strand breaks fail to repair[51]. Mutation of *p53* is one of the most prevalent genetic alterations in human cancer, including gastric carcinoma. The gene is usually inactivated through the classic two-hit mechanism, i.e. LOH and mutation of the remaining allele, rather than by DNA methylation[52]. The frequency of *p53* mutations in early and advanced differentiated gastric carcinomas is consistent at around 40% each, similar to that observed for advanced undifferentiated carcinomas[53,54]. However, *p53* mutations are rare in early undifferentiated carcinomas[17,55]. Thus, *p53* gene mutation is thought to be an early event, critical in the development of differentiated carcinomas, and the frequent detection of *p53* mutations in advanced undifferentiated carcinomas is postulated to be due to the frequent conversion of differentiated cancers to an undifferentiated phenotype as the tumors progress[50].

hMLH1

Epigenetic methylation-associated inactivation of the *hMLH1* mismatch repair gene is a potent trigger of MSI, especially high-frequency MSI (MSI-H)[56]. Since the first report of *hMLH1* inactivation associated with DNA methylation in colorectal cancer[56], similar epigenetic alterations have been described in gastric cancer[11,13,16]. DNA methylation of *hMLH1* promoter region CpG island is tightly associated with the loss of hMLH1 expression in gastric cancers exhibiting MSI[11,13,16]. About 20% of early differentiated carcinomas exhibit MSI-H[47], while early undifferentiated carcinomas show no evidence of MSI (as described below)[17]. *hMLH1* methylation is frequently observed in gastric cancers from elderly patients[31] and has also been described in non-neoplastic gastric epithelia surrounding gastric cancers with MSI[16,57]. Thus, this field defect may increase the risk of subsequent neoplasia as MSI-H has also been observed in patients with multiple gastric cancers[58].

E-cadherin

E-cadherin is a member of a family of transmembrane glycoproteins involved in calcium-dependent cell-to-cell adhesion and appears to play a role in organogenesis and morphogenesis[59]. Germline *E-cadherin* mutations have been reported in familial diffuse-type of gastric cancers[5]. *E-cadherin* is frequently inactivated via the classic two-hit mechanism in sporadic forms of undifferentiated-scattered (diffuse) type gastric carcinomas, but not in differentiated or undifferentiated adherent type gastric carcinomas[2,3]. While nearly half of the undifferentiated-scattered (diffuse) type gastric carcinomas contain *E-cadherin* mutations[2,3], such mutations are rare in early undifferentiated carcinomas[3,60], and are only detected in the undifferentiated component of mixed differentiated/undifferentiated carcinomas[61]. This suggests that *E-cadherin* mutations are involved in the de-differentiation of such tumors. In contrast, *E-cadherin* methylation, which is associated with decreased E-cadherin expression, is observed in >50% of early stage undifferentiated carcinomas[6,17], and is also observed in surrounding non-cancerous gastric epithelia[14,31]. Thus, the epigenetic inactivation of *E-cadherin via* promoter methylation may play a major role in the development of purely undifferentiated carcinomas of the stomach, while mutation of the gene may lead to the de-differentiation of differentiated gastric tumors.

Other tumor suppressor genes

APC gene mutation is a critical genetic event in both the familial and sporadic forms of colorectal tumorigenesis[62,63]. *APC* mutations are rare in extracolonic cancers, including gastric carcinomas, with less than 10% of both differentiated and undifferentiated gastric carcinomas containing such mutations[17,46,54,64]. While *APC* promoter methylation has also been reported in colorectal and other human neoplasms[65], *APC* methylation (promoter 1A) does not appear to be oncogenic in gastric cancer[18]. Mutation and promoter methylation of *DCC,*

p16, and *PTEN* genes have also been investigated in gastric cancer[14,23,24,66]. Although few mutations in these genes have been found, the promoter regions of *DCC* and *p16*, but not *PTEN*, exhibit frequent methylation, suggesting that epigenetic inactivation of *DCC* and *p16* may be involved in gastric carcinogenesis[14,23]. *DAP-kinase* promoter methylation is more frequent in undifferentiated than in differentiated type tumors[21,30,32]. While *RASSF1A* gene mutations are uncommon, silencing of the gene by promoter methylation is frequent in carcinomas, including gastric carcinomas[19,67]. *RUNX3*, one of the three mammalian runt-related genes, was recently identified as a tumor suppressor gene that frequently shows loss of expression due to hemizygous deletion and hypermethylation in gastric cancer[27]. *RUNX3* methylation is mostly cancer-specific, and is observed in about half of all gastric cancer cases[32]. *TSLC1* has been shown to be inactivated by biallelic methylation in a proportion of primary gastric cancers[25]. *CHFR* hypermethylation is found to occur concurrently with *hMLH1* hypermethylation and is more frequent in patients over 70 years of age[68].

Thus, many tumor suppressor and tumor-related genes are methylated in neoplastic and non-neoplastic gastric epithelia, although the significance of hypermethylation is dependent on the analyzed genomic region[69]. In non-neoplastic gastric epithelia, hypermethylation tends to initially occur in the 5'- and 3'-flanking regions of CpG islands and then spreads toward the transcription start site, whereupon protein expression is shut down. This ultimately results in a field defect that places the affected tissue at an increased risk of gastric cancer development[29]. Hypermethylation near a transcription start site, which can be cancer-specific and result in gene silencing, can be used as a diagnostic marker of malignancy in tissues or other samples, such as serum or ascites. In addition, hypermethylation at a region next to such a critical region might indicate an early signal of carcinogenesis.

LOH

In differentiated carcinomas of the stomach, frequent LOH has been reported for several chromosomal arms, including 2q, 4p, 5q, 6p, 7q, 11q, 14q, 17p, 18q and 21q[7,70-73]. However, few reports have focused on the occurrence of LOH in undifferentiated carcinomas, probably due to the difficulty in performing LOH analysis on tissue samples with low tumor cellularity. Nonetheless, frequent LOH at 5q has been reported for both differentiated and undifferentiated tumor types at advanced stages[74,75]. Apart from a few exceptions, such as the *p53* gene on 17p, the target suppressor gene(s) in the LOH regions on these chromosomal arms remain(s) largely unknown. For example, *IRF-1* on 5q31.1 and *DPC4* (*Smad4*) on 18q21.1 are both located at commonly deleted regions identified in gastric cancer, but exhibit infrequent mutations in gastric cancer[70,75,76]. The methylation status of the *IRF-1* and *DPC4* (*Smad4*) gene promoter regions remains to be investigated.

MSI

MSI is defined as the presence of replication errors in simple repetitive microsatellite sequences due to defective DNA mismatch repair, and can be classified as either high-frequency (MSI-H), low-frequency (MSI-L) or stable (MSS)[77]. The prevalence of MSI in gastric cancer varies among different studies. While some reports suggest that differentiated carcinomas exhibit more frequent MSI than undifferentiated carcinomas[78], other reports observe the opposite findings[79]. Again, these contradictory observations may be due to the frequent conversion of differentiated- to undifferentiated-type tumors[50], as described for *p53* mutations. In a study where MSI analysis was restricted to early differentiated carcinomas (ordinary type), about 20% of tumors were classified as MSI-H[47]. In contrast, no evidence of MSI has been found in early undifferentiated carcinomas[17]. Gastric cancers with an MSI phenotype rarely exhibit structural alterations, such as mutations or LOH of tumor suppressor genes[46,47,80], which suggests that the mutator and suppressor pathways are independent of each other at least in the early stages of gastric carcinogenesis.

Promoter demethylation of MAGE and synuclein-γ

Melanoma antigen (MAGE)-encoding genes are expressed in various tumor types via demethylation of their promoter CpG islands, which are silent in all non-neoplastic tissues except for the testis and placenta. While the function of the MAGE peptides is not known, their tumor-specific expression is clearly of great significance to immunotherapy[81-83]. Demethylation of both the *MAGE-A1* and *-A3* promoters is more frequently observed in gastric cancer patients with advanced clinical stages. These patients also exhibit a higher incidence of lymph node metastasis compared to patients without demethylation[41]. Furthermore, patients exhibiting *MAGE-A1* and *-A3* promoter demethylation tend to have a worse prognosis, as assessed by the log rank test[41]. Demethylation of *MAGE-A1* and *-A3* tends to occur during the progressive stages of gastric cancer, and may therefore act as a prognostic factor for gastric cancer patients.

The *synuclein-γ* (*SNCG*) gene, also known as *breast cancer specific gene 1* (*BCSG1*), is a member of the synuclein neuronal protein family, along with *synuclein-α* (*SNCA*) and *synuclein-β* (*SNCB*)[84-86]. SNCG protein expression is highly tissue-specific, being expressed at presynaptic terminals in the brain and peripheral nervous system[85,86]. However, this tissue specificity is lost during breast and ovarian cancer disease progression[87]. While *SNCG* expression is normally silent in the breast and ovary, it becomes abundantly expressed in the vast majority of advanced-stage breast and ovarian cancers[87]. *SNCG* demethylation is also found to be more frequent in primary gastric cancers positive for lymph node metastasis than in metastasis-negative cancers, and more frequent in stage II-IV cancers than in stage I cancers[42]. An increased tendency for gastric cancer patients with poor prognoses to show *SNCG* demethylation compared to gastric cancer patients with normal methylation has also been reported[42].

Global DNA hypomethylation is thought to occur during the early stages of tumor development in gastric and other tissues[37-40]. However, *MAGE-A1*

and *-A3* demethylation are very rare in various organs obtained at autopsy from various age groups[41], and only partial demethylation of *SNCG* is present in non-neoplastic gastric epithelia[42]. Therefore, we hypothesize that demethylation of these genes occurs during the progressive stages of gastric carcinogenesis, after global DNA hypomethylation.

GENETIC AND EPIGENETIC ALTERATIONS IN PRECANCEROUS LESIONS

Gastric adenoma/dysplasia

The histopathologic criteria for the diagnosis of gastric intramucosal neoplasia are not universal, and differences in the diagnostic criteria used by Japanese and Western pathologists have been recognized[88]. It is reasonable to suggest that the discrepant results obtained from the genetic analyses of lesions may be explained by the differences in histopathologic criteria, although a worldwide accepted histological classification has recently been proposed[89,90]. In my experience, gastric adenomas rarely exhibit genetic alterations, such as *p53* mutation, LOH, or MSI[10,13,54], with mutations of *APC* gene being the only relatively frequent (20%) DNA structural alteration[91]. Indeed, *APC* gene mutations are more frequent in gastric adenomas than in differentiated or undifferentiated gastric carcinomas[17,46,54,64]. More recently, we reported that results of the Padova international classification[89] correlated with both molecular and cellular phenotypic profiles, and that p53 and hMLH1 immunohistochemistry clearly discriminated these lesions[92]. Histopathologic observations have suggested that malignant transformation of gastric adenomas is infrequent, occurring in only 2.5% of conventional protruded and 5.0% of depressed adenomas[93]. However, detection of certain genetic alterations, such as *p53* mutations, LOH, or MSI, in adenomas may be indicative of malignant transformation[94]. It is noteworthy that gastric-type intramucosal neoplasia, often diagnosed as adenoma or dysplasia[95], frequently shows a mutator defect[16].

Gastric intestinal metaplasia/non-neoplastic gastric epithelia

Intestinal metaplasia may be a precursor of differentiated carcinomas. This concept is supported by the finding that *p53* mutations are detected in gastric intestinal metaplasia, especially incomplete-type, in patients with gastric cancer[96]. Although frequent MSI has been reported in intestinal metaplasia[97], there is little evidence of mismatch repair defects in this tissue[98]. *Helicobacter pylori* infection can accelerate the hypermethylation of genes such as *E-cadherin*[99]. Hypermethylation of tumor suppressor and tumor-related genes increases with age, and is thought to result in field defects in different organs[100], although the significance of the hypermethylation appears to be dependent on the genomic region analyzed[69], as described above for gastric cancer.

CONCLUSIONS

The molecular pathways of gastric carcinogenesis are dependent on the histological background, such that different genes are affected in different histologies. DNA structural alterations, including *p53* gene mutation and LOH, occur predominantly within intestinal metaplastic mucosa. Hypermethylation of tumor suppressor and tumor-related genes can occur in both metaplastic and native gastric epithelial cells, although at least some of the genes involved, such as *E-cadherin*, are more prone in the latter. Approximately 20% of differentiated carcinomas display evidence of mutator pathway tumorigenesis due to *hMLH1* hypermethylation. Demethylation of *MAGE* and *synuclein-γ* tends to occur during progressive disease stages. Thus, these genetic and epigenetic alterations can be used in the risk assessment, diagnosis and prognosis of gastric cancer.

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