Summary. Both genetic and epigenetic alterations of tumor suppressor and tumor-related genes involved in the pathogenesis of gastric cancer are reviewed here, and molecular pathways of gastric carcinogenesis are proposed. Gastric carcinomas are believed to evolve from native gastric mucosa or intestinal metaplastic mucosa that undergoes genetic and epigenetic alterations involving either the suppressor pathway (defects in tumor suppressor genes) or mutator pathway (defects in DNA mismatch repair genes). Methylation of E-cadherin in native gastric mucosa results in undifferentiated carcinomas (suppressor pathway), while methylation of hMLH1 results in differentiated foveolar-type carcinomas (mutator pathway). The majority of differentiated gastric carcinomas however, arise from intestinal metaplastic mucosa and exhibit structural alterations of tumor suppressor genes, especially p53. They appear to be related to chronic injury, perhaps due to Helicobacter pylori infection. Approximately 20% of differentiated carcinomas (ordinary-type) have evidence of mutator pathway tumorigenesis. Mutations of E-cadherin are mainly involved in the progression of differentiated carcinomas to undifferentiated tumors. The molecular pathways of gastric carcinogenesis depend on the histological background, and gastric carcinomas show distinct biological behaviors as a result of discernible cellular genetic and epigenetic alterations.

Key words: Gastric cancer, p53, E-cadherin, hMLH1, Methylation

Introduction

Since the initial report of frequent mutations of the p53 tumor suppressor gene in primary gastric cancers (Tamura et al., 1991), a series of genetic and epigenetic alterations of tumor suppressor and tumor-related genes involved in gastric carcinogenesis have been identified. These alterations may be divided into two groups: alterations resulting in tumor suppressor gene inactivation (suppressor pathway); and those resulting in DNA mismatch repair gene deficiency (mutator pathway, Perucho, 1996). Tumor suppressor and tumor-related genes can be inactivated not only by the classic two-hit mechanism but also by combinations of gene mutation, loss of heterozygosity (LOH), or DNA methylation (Jones and Laird, 1999). DNA methylation of promoter region CpG islands inhibits gene transcription by interfering with transcription initiation, and serves as an alternative mechanism to coding region mutations in inactivating tumor suppressor and tumor-related genes (Jones and Laird, 1999).

With regard to the suppressor pathway, mutations of the tumor suppressor genes p53 and epithelial (E)-cadherin are frequent and important (Tamura et al., 1991, 1996d; Becker et al., 1994). In addition, epigenetic inactivation of E-cadherin, p16, and DCC has also been described in gastric cancer (Suzuki et al., 1999; Tamura et al., 2000; Sato et al., 2001a). Frequent LOH, a surrogate marker of the presence of tumor suppressor gene(s) within a deleted region, has been found on several chromosomal arms (Tamura et al., 1996e). However, the tumor suppressor genes isolated from these regions thus far, (e.g., IRF-1 on 5q31.1 and DPC4 (Smad4) on 18q21.1), exhibit infrequent mutations in gastric cancer (Nishizuka et al., 1997a; Nozawa et al., 1998).

Mutations in the DNA mismatch repair genes, hMSH2 and hMLH1, members of the mutator pathway, are rare (Akiyama et al., 1996; Semba et al., 1998) despite the finding of frequent microsatellite instability (MSI) in gastric cancers (Tamura et al., 1995). More recently, methylation of the hMLH1 gene promoter has been found to be responsible for the development of the majority of microsatellite-unstable gastric cancers (Fleisher et al., 1999). Inactivation of hMLH1 via promoter methylation leads to MSI, and subsequently to mutations in simple repetitive sequences of 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 (Kim et al., 1999).

From a histopathological point of view, gastric cancers are classified as either differentiated carcinoma, which form tubular or papillary structures (roughly corresponding to an intestinal type), or undifferentiated carcinomas in which such structures are inconspicuous (roughly corresponding to the diffuse type) (Lauren, 1965; Nakamura et al., 1968). Differentiated carcinomas, with a predominantly intestinal cellular phenotype, were thought to originate from gastric epithelial cells that subsequently underwent intestinal metaplasia, while undifferentiated carcinomas arose from native gastric epithelial cells (Lauren, 1965; Nakamura et al., 1968; Jass and Filipe, 1981). Recent advances in mucin histochemistry and immunohistochemistry, however, indicate that some differentiated carcinomas have a predominantly (and, on occasion exclusively) gastric cellular phenotype, which appears to be derived from foveolar epithelial cells (Endoh et al., 2000a; Ohmura et al., 2000). In addition, gastric cancers appear to undergo changes in their cellular phenotype, from gastric to intestinal, over time (Tatematsu et al., 1992). Thus, differentiated carcinomas may develop from native gastric mucosa or intestinal metaplastic mucosa. Although different genetic pathways have been proposed for both differentiated and undifferentiated histological types (Tahara, 1995), they must in fact share some common genetic alterations as a significant proportion of differentiated carcinomas progress to undifferentiated tumors (Endoh et al., 2000c). Recent studies moreover, indicate that the tumor cell phenotype is more a marker of particular genetic aberrations (Endoh et al., 2000a; Ohmura et al., 2000). In relation to undifferentiated gastric cancers, our laboratory has recently identified early molecular features of these tumors (Tamura et al., 2001).

In this article, I review genetic and epigenetic alterations involved in the pathogenesis of gastric cancers, and molecular pathways of gastric carcinogenesis are proposed. Genetic and epigenetic alterations observed in precancerous lesions (gastric adenoma/dysplasia, Rugge et al., 2000) and their potential application to the molecular diagnosis of these lesions is described.

Genetic and epigenetic alterations of tumor suppressor genes: suppressor pathway

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 G1 arrest in the presence of DNA damage and inducing DNA repair genes, and initiates apoptosis of cells that fail to repair DNA strand breaks (Levine, 1997). Mutation of p53 is one of the most prevalent genetic alterations in human cancers including gastric carcinoma. The gene is inactivated through the classic two-hit mechanism, i.e. LOH and mutations of the remaining allele, but not by means of DNA methylation (Jones and Laird, 1999). The frequency of p53 mutations in early and advanced differentiated gastric carcinomas is consistent at around 40%, similar to that observed in advanced undifferentiated carcinomas (UCHINO et al., 1993; MAESAWA et al., 1995). However, p53 mutations are rare in early undifferentiated carcinomas (RANZANI et al., 1995; TAMURA et al., 2001). Thus, mutations of the p53 gene are considered to be critical early events in the development of differentiated carcinomas, and the frequent detection of p53 mutations observed in advanced undifferentiated carcinomas is postulated to be due to the frequent conversion of differentiated cancers to an undifferentiated phenotype as these tumors progress (Endoh et al., 2000c).

E-cadherin

E-cadherin is a member of a family of transmembrane glycoproteins responsible for calcium-dependent cell-to-cell adhesion and appears to play a role in organogenesis and morphogenesis (Takeichi, 1991). Germline E-cadherin mutations have been reported in diffuse-type gastric cancer families (Guilford et al., 1998; Gayther et al., 1999). In addition, 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 (Becker et al., 1994; Tamura et al., 1996d). Nearly half of undifferentiated-scattered (diffuse) type gastric carcinomas have evidence of E-cadherin mutations (Becker et al., 1994; Tamura et al., 1996d). Such mutations, however, are rare in early undifferentiated carcinomas (Muta et al., 1996; Tamura et al., 1996d). In addition, E-cadherin mutations are only detected in the undifferentiated component of mixed differentiated/undifferentiated carcinomas (MACHADO et al., 1999), suggesting that E-cadherin mutations allow dedifferentiation of these tumors. In contrast, E-cadherin methylation, which is associated with decreased E-cadherin expression, is observed in >50% of early stage undifferentiated carcinomas (Tamura et al., 2000, 2001), and is also observed in the surrounding non-cancerous gastric epithelia (Suzuki et al., 1999). Thus, epigenetic inactivation of E-cadherin via promoter methylation may play a major role in the development of purely undifferentiated carcinomas of the stomach, while mutations of the gene may lead to dedifferentiation of differentiated gastric tumors.

Other tumor suppressor genes

Mutations of APC are critical genetic events in both familial and sporadic forms of colorectal tumorigenesis (MIYOSHI et al., 1992a,b). APC mutations are rare in extracolonic cancers including gastric carcinomas, and less than 10% of both differentiated and undifferentiated
gastric carcinomas have evidence of these mutations (Hori et al., 1992; Maesawa et al., 1995; Endoh et al., 2000a; Tamura et al., 2001). Promoter methylation of APC has also been reported in colorectal and other human neoplasms (Esteller et al., 2000); however, APC methylation does not appear to be oncogenic in gastric cancer (Tsuchiya et al., 2000). Mutations and promoter methylation of DCC, p16, and PTEN have also been investigated in gastric cancer (Sakata et al., 1995; Suzuki et al., 1999; Sato et al., 2001a,b). Few mutations of these genes were found. However, the promoter regions of DCC and p16, but not PTEN exhibited frequent methylation, suggesting possible involvement of DCC and p16, by epigenetic inactivation, in gastric carcinogenesis (Suzuki et al., 1999; Sato et al., 2001a).

**Loss of heterozygosity (LOH)**

LOH has been frequently reported for several chromosomal arms, including 2q, 4p, 5q, 6p, 7q, 11q, 14q, 17p, 18q, and 21q, in differentiated carcinomas of the stomach (Tamura et al., 1996c; Nishizuka et al., 1997b, 1998; Sakata et al., 1997). However, few reports have focused on LOH in undifferentiated carcinomas, probably due to the difficulty of performing LOH analysis on tissue samples with low tumor cellularity. Nonetheless, frequent LOH of 5q has been reported for both of these tumor types in their advanced stages, with or without tumor cell enrichment (Mckie et al., 1993; Tamura et al., 1993). The target suppressor gene(s) in the LOH regions on these chromosomal arms are largely unknown, apart from p53 on 17p. For example, IRF-1 on 5q31.1 and DPC4 (Smad4) on 18q21.1, both located at commonly deleted regions identified in gastric cancer (Tamura et al., 1996c; Nishizuka et al., 1997a), exhibited infrequent mutations in gastric cancers (Nishizuka et al., 1997a; Nozawa et al., 1998). The methylation status of the promoter regions of the IRF-1 and DPC4 (Smad4) genes remains to be investigated.

**Microsatellite instability (MSI) and hMLH1 promoter methylation: mutator pathway**

**MSI**

MSI is defined as the presence of replication errors in simple repetitive microsatellite sequences due to DNA mismatch repair deficiency, and is classified as either high-frequency (MSI-H), low-frequency (MSI-L) or stable (MSS) (Boland et al., 1998). The frequency of MSI in gastric cancer varies in different studies. Some reports suggest that differentiated carcinomas exhibit more frequent MSI than undifferentiated (Tamura et al., 1997), while others observed the opposite findings (Han et al., 1993). Again, these contradictory observations may be due to the frequent conversion of differentiated tumors to an undifferentiated type (Endoh et al., 2000c), as described above for p53 mutations. In a study where MSI analysis was restricted to early differentiated carcinomas (ordinary-type), 19% (10/52) were classified as MSI-H, 12% (6/52) as MSI-L, and 69% (36/52) as MSS (Ohmura et al., 2000). In contrast, none of the early undifferentiated carcinomas had evidence of MSI (Tamura et al., 2001). Gastric cancers with an MSI phenotype rarely exhibit structural alterations (mutations or LOH) of tumor suppressor genes (Endoh et al., 2000a; Ohmura et al., 2000; Ogata et al., 2001), suggesting that the mutator and suppressor pathways are independent of each other at least in the early stages of gastric carcinogenesis.

**hMLH1 methylation**

Since inactivation of hMLH1 in association with DNA methylation was first reported in colorectal cancer (Herman et al., 1998), similar epigenetic alterations have been described in gastric cancer (Fleisher et al., 1999, 2001; Endoh et al., 2000b). DNA methylation of hMLH1 promoter region CpG islands is tightly associated with loss of hMLH1 expression in gastric cancers exhibiting MSI (Fleisher et al., 1999, 2001; Endoh et al., 2000b). Furthermore, hMLH1 methylation has also been described in non-cancerous gastric epithelia that surrounds gastric cancers exhibiting MSI (Endoh et al., 2000b; Guo et al., 2001). Such a field defect may increase the risk of subsequent neoplasia as a high frequency of MSI has been observed in patients with multiple gastric cancers (Nakashima et al., 1995).

**Molecular pathways of gastric carcinogenesis**

**Cellular phenotype**

Although the cellular phenotype of gastric cancers may alter as the tumor progresses (Tatematsu et al., 1992), early cancers most likely retain evidence of their histological origin. Using a strict definition of cellular phenotype, differentiated carcinomas have been classified into four groups: gastric phenotype (foveal epithelial and pyloric gland phenotype); complete-type intestinal metaplastic phenotype (CIM-type); ordinary phenotype (including incomplete-type intestinal phenotype); and unclassified phenotype (Ohmura et al., 2000). Although the foveolar-type and CIM-type carcinomas constitute less than 10% of all early differentiated carcinomas, tight linkage has been shown between these cellular phenotypes and specific genetic pathways in differentiated carcinomas (Fig. 1) (Ohmura et al., 2000), suggesting that differentiated carcinomas may arise from both foveolar epithelial cells and intestinal metaplastic epithelial cells.

**Molecular pathways and the histogenesis of gastric cancer**

Undifferentiated carcinomas and differentiated foveolar-type carcinomas, both of which may arise from native gastric mucosa, frequently exhibit DNA
methylation of either E-cadherin or hMLH1, respectively. These tumors, at least during their early stages, rarely exhibit structural alterations of tumor suppressor genes (Endoh et al., 2000a; Ohmura et al., 2000; Tamura et al., 2001). Methylation of the E-cadherin gene in foveolar epithelial cells may lead to undifferentiated carcinomas (suppressor pathway), while methylation of hMLH1 in the same cell type may result in differentiated foveolar-type carcinomas (mutator pathway). It is noteworthy, however, that a subgroup of undifferentiated gastric carcinoma observed in younger patients, appears to be due to mutations of the E-cadherin gene (Saito et al., 1999). As age-related methylation has the potential to behave as a mutator process resulting in silencing of multiple tumor suppressor and tumor-related genes in aging tissues (Issa, 2000), these tumors may also arise from foveolar epithelial cells through this process. In contrast, the majority of ordinary type and CIM-type carcinomas, which are presumed to arise from intestinal metaplastic cells, show structural alterations of tumor suppressor genes, especially p53. These tumors, representative of tumorigenesis via the suppressor pathway are associated with chronic injury to the gastric mucosa, which may be related to Helicobacter pylori infection (Schmidt et al., 1999). Approximately 20% of ordinary-type carcinomas have evidence of mutator pathway tumorigenesis (Ohmura et al., 2000). These observations have led the present author to propose putative molecular pathways of the development of gastric carcinoma in relation to histogenesis (Fig. 2).

**Genetic and epigenetic alterations in precancerous lesions**

**Gastric adenoma/dysplasia**

The histopathological criteria for the diagnosis of gastric intramucosal neoplasia are not universal, and differences in diagnostic criteria between Japanese and western pathologists have been recognized (Schlemper et al., 1997). Although a worldwide-accepted histological classification has been proposed recently (Rugge at al., 2000), it is reasonable to suggest that controversial results of genetic analyses of such lesions may derive from these differences in histopathological criteria (Tamura et al., 1996a). In the experience of this author, gastric adenomas rarely exhibit genetic alterations, such as p53 mutation, LOH, or MSI (Maesawa et al., 1995; Tamura et al., 1995; Fleisher et al., 2001). Mutations of the APC gene are the only DNA structural alterations that are relatively frequent (20%) in gastric adenomas (Tamura et al., 1994). In fact, mutations of the APC gene are more frequent in gastric adenomas than in either differentiated or undifferentiated gastric carcinomas (Horii et al., 1992; Maesawa et al., 1995; Endoh et al., 2000a; Tamura et al., 2001). Histopathological observations would suggest that malignant transformation of gastric adenoma occurs infrequently, occurring in 2.5% of conventional protruded and 5.0% of depressed adenomas (Nakamura et al., 1988). However, detection of certain genetic alterations, such as p53 mutations, LOH, or MSI, in adenomas may suggest the presence of malignant transformation (Tamura et al., 1996b). It is noteworthy that gastric-type intramucosal neoplasia, often diagnosed as adenoma or dysplasia (Kushima et al., 1996), frequently shows a mutator defect (Endoh et al., 2000b).

**Gastric intestinal metaplasia**

Intestinal metaplasia may be a precursor of differentiated carcinoma. This concept is supported by the finding that mutations of p53 are detected in gastric intestinal metaplasia, especially of the incomplete type, in patients with gastric cancer (Ochiai et al., 1996). Although frequent MSI has been reported in intestinal metaplasia (Leung et al., 2000), there is little evidence of mismatch repair defects in this tissue (Jin et al., 2001). Although hMLH1-methylated cells may be present in non-cancerous gastric epithelia, it is not clear which cell
type in intestinal metaplastic mucosa is affected by this epigenetic phenomenon.

Application to molecular diagnosis

As gastric intramucosal lesions can present a diagnostic dilemma, the availability of objective diagnostic markers is desirable. Genetic and epigenetic alterations that involve the suppressor and mutator pathways, which can be detected in biopsy specimens, have been employed as candidate diagnostic markers. In practice, determination of altered protein expression of p53 (a marker of a suppressor pathway defect) and hMLH1 (a marker of a mutator pathway defect) serve as simple and useful markers, as about 70% of intramucosal differentiated carcinomas show abnormalities of one or other of these proteins, while adenoma/dysplasia exhibit few such abnormalities (unpublished data). In addition, detection of either a suppressor or mutator pathway defect is useful in therapeutic decisions, because gastric carcinomas with a mutator pathway defect show less frequent lymph node metastasis and a more favorable prognosis in comparison to those tumors with a suppressor pathway defect (dos Santos et al., 1996; Wu et al., 2000). The presence of cells with methylation of the hMLH1 gene promoter within non-cancerous gastric mucosa may be associated with an increased risk of multiple gastric cancer (Sakata et al., 2002). Interestingly, promoter methylation of tumor suppressor and tumor-related genes are observed also in non-cancerous gastric mucosa at variable frequencies in which methylation has been detected in gastric cancers (Table 1). Two possible explanations should be considered. Firstly, methylation of genes in normal (non-cancerous) tissue is an age-related phenomenon and not oncogenic, e.g., APC methylation (Tsuchiya et al., 2000), or conversely, it is precancerous and represents a field defect as has been suggested in the case of hMLH1 methylation (Sakata et al., 2002). APC mutations are the most frequent genetic alterations detected in gastric adenomas (Tamura et al., 1994). However, the significance of their detection remains uncertain because of their rarity in gastric carcinoma (Horii et al., 1992; Maesawa et al., 1995; Endoh et al., 2000a; Tamura et al., 2001).

Conclusions

The molecular pathways of gastric carcinogenesis depend on the histological background. Methylation in E-cadherin and hMLH1 occurs in native gastric epithelial cells. DNA structural alterations including gene mutation occur predominantly within intestinal metaplastic epithelial cells during inflammatory and regenerative processes. Importantly, gastric carcinomas show distinct biological behaviors as a result of discernible cellular genetic and epigenetic alterations.

Table 1. Promoter methylation in normal and cancerous gastric epithelia.

<table>
<thead>
<tr>
<th>GENE</th>
<th>PROMOTER METHYLATION</th>
<th>Normal</th>
<th>Cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-Cadherin</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>hMLH1</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>p16</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>DCC</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>PTEN</td>
<td>No</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>APC</td>
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<td>No</td>
<td></td>
</tr>
<tr>
<td>promoter 1A</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>promoter 1B</td>
<td>No</td>
<td>No</td>
<td></td>
</tr>
</tbody>
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