

Review

Genetic and epigenetic alterations of tumor suppressor and tumor-related genes in gastric cancer

G. Tamura

Department of Pathology, Yamagata University School of Medicine, Yamagata, Japan

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 (Horii 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,e; 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 (Yamamoto et al., 1999), 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 (foveolar 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 et 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

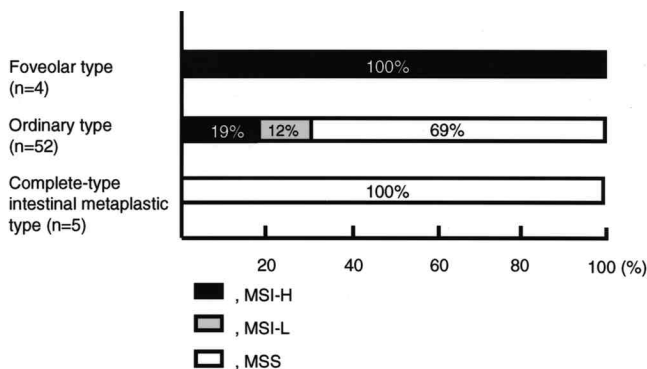


Fig. 1. The frequency of microsatellite instability in early differentiated carcinomas of the foveolar type (n=4), ordinary type (n=52), and complete-type intestinal metaplastic type (n=5) (Ohmura et al., 2000).

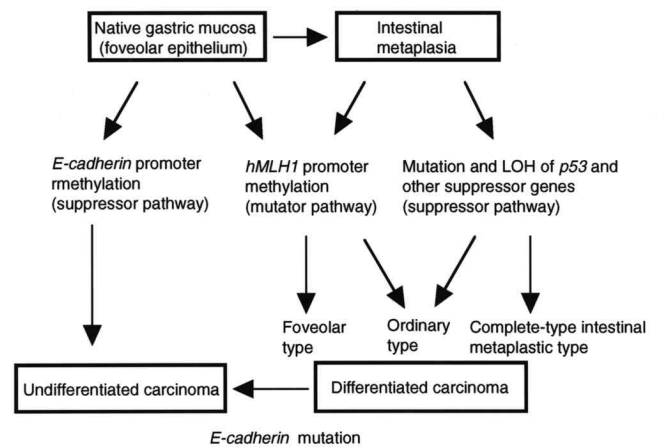


Fig. 2. Molecular pathways of gastric carcinogenesis

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).

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

GENE	PROMOTER METHYLATION	
	Normal	Cancer
<i>E-Cadherin</i>	Yes	Yes
<i>hMLH1</i>	Yes	Yes
<i>p16</i>	Yes	Yes
<i>DCC</i>	Yes	Yes
<i>PTEN</i>	No	No
<i>APC</i> promoter 1A	Yes	Yes
promoter 1B	No	No

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.

References

- Akiyama Y., Nakasaki H., Nihei Z., Iwama T., Nomizu T., Utsunomiya J. and Yuasa Y. (1996). Frequent microsatellite instabilities and analyses of the related genes in familial gastric cancers. *Jpn. J. Cancer Res.* 87, 595-601.
- Becker K.F., Atkinson M.J., Reich U., Becker I., Nekarda H., Siewert J.R. and Hofler H. (1994). *E-cadherin* gene mutations provide clues to diffuse type gastric carcinomas. *Cancer Res.* 54, 3845-3852.
- Boland C.R., Thibodeau S.N., Hamilton S.R., Sidransky D., Eshleman J.R., Burt R.W., Meltzer S.J., Rodriguez-Bigas M.A., Fodde R., Ranzani G.N. and Srivastava S. (1998). A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. *Cancer Res.* 58, 5248-5257.
- dos Santos N.R., Seruca R., Constancia M., Seixas M. and Sobrinho-Simoes M. (1996). Microsatellite instability at multiple loci in gastric carcinoma: clinicopathologic implications and prognosis. *Gastroenterology* 110, 38-44.
- Endoh Y., Sakata K., Tamura G., Ohmura K., Ajioka Y., Watanabe H. and Motoyama T. (2000a). Cellular phenotypes of differentiated-type adenocarcinomas and precancerous lesions of the stomach are dependent on the genetic pathways. *J. Pathol.* 191, 257-263.
- Endoh Y., Tamura G., Watanabe H., Ajioka Y. and Motoyama T. (2000b). Frequent hypermethylation of the *hMLH1* gene promoter in differentiated-type tumors of the stomach with the gastric foveolar phenotype. *Am. J. Pathol.* 157, 717-722.
- Endoh Y., Tamura G., Watanabe H. and Motoyama T. (2000c). Author's reply. *J. Pathol.* 191, 467-468.
- Esteller M., Sparks A., Toyota M., Sanchez-Cespedes M., Capella G., Peinado M.A., Gonzalez S., Tarafa G., Sidransky D., Meltzer S.J., Baylin S.B. and Herman J.G. (2000). Analysis of adenomatous polyposis coli promoter hypermethylation in human cancer. *Cancer Res.* 60, 4366-4371.
- Fleisher A.S., Esteller M., Wang S., Tamura G., Suzuki H., Yin J., Zou T-T., Abraham J.M., Kong D., Smolinski K.N., Shi Y-Q., Rhyu M-G., Powell S.M., James S.P., Wilson K.T., Herman J.G. and Meltzer S.J. (1999). Hypermethylation of the *hMLH1* gene promoter in human gastric cancers with microsatellite instability. *Cancer Res.* 59, 1090-1095.
- Fleisher A.S., Esteller M., Tamura G., Rashid A., Stine O.C., Yin J., Zou T-T., Abraham J.M., Kong D., Nishizuka S., James S.P., Wilson K.T., Herman J.G. and Meltzer S.J. (2001). Hypermethylation of the *hMLH1* gene promoter is associated with microsatellite instability in early human gastric neoplasia. *Oncogene* 20, 329-335.
- Gayther S.A., Goringe K.L., Ramus S.J., Huntsman D., Roviello F.,

- Grehan N., Machado J.C., Pinto E., Seruca R., Halling K., MacLeod P., Powell S.M., Jackson C.E., Ponder B.A. and Caldas C. (1999). Identification of germ-line *E-cadherin* mutation in gastric cancer families of European origin. *Cancer Res.* 58, 4086-4089.
- Guilford P., Hopkins J., Harraway J., McLeod M., McLeod N., Harawira P., Taite H., Scouler R., Miller A. and Reeve A.E. (1998). *E-cadherin* germline mutations in familial gastric cancer. *Nature (Lond.)* 392, 402-405.
- Guo R.J., Arai H., Kitayama Y., Igarashi H., Hemmi H., Arai T., Hanai H. and Sugimura H. (2001). Microsatellite instability of papillary subtype of human gastric adenocarcinoma and *hMLH1* promoter hypermethylation in the surrounding mucosa. *Pathol. Int.* 51, 240-247.
- Han H.J., Yanagisawa A., Kato Y., Park J.G. and Nakamura Y. (1993). Genetic instability in pancreatic cancer and poorly differentiated type of gastric cancer. *Cancer Res.* 53, 5087-5089.
- Herman J.G., Umar A., Polyak K., Graff J.R., Ahuja N., Issa J-P.J., Markowitz S., Willson J.K.V., Hamilton S.R., Kinzler K.W., Kane M. F., Kolodner R.D., Vogelstein B., Kunkel T.A. and Baylin S.B. (1998). Incidence and functional consequence of *hMLH1* promoter hypermethylation in colorectal carcinoma. *Proc. Natl. Acad. Sci. USA* 95, 6870-6875.
- Horii A., Nakatsuru S., Miyoshi Y., Ichii S., Nagase H., Kato Y., Yanagisawa A. and Nakamura Y. (1992). The *APC* gene, responsible for familial adenomatous polyposis, is mutated in human gastric cancer. *Cancer Res.* 52, 3231-3233.
- Issa J.P. (2000). CpG-island methylation in aging and cancer. *Curr. Top. Microbiol. Immunol.* 249, 101-118.
- Jass J.R. and Filipe M.I. (1981). The mucin profiles of normal gastric mucosa, intestinal metaplasia and its variants and gastric carcinoma. *Histochem. J.* 13, 931-939.
- Jin Z., Tamura G., Satoh M., Meguro T., Miura T., Hayashi M., Osakabe M., Ohmura K., Ogata S., Endoh Y. and Motoyama T. (2001). Absence of *BAT-26* instability in gastric intestinal metaplasia. *Pathol. Int.* 51, 473-475.
- Jones P.A. and Laird P.W. (1999). Cancer epigenetics comes of age. *Nat. Genet.* 21, 63-67.
- Kim J.J., Baek M.J., Kim L., Kim N.G., Lee Y.C., Song S.Y., Noh S.H. and Kim H. (1999). Accumulated frameshift mutations at coding nucleotide repeats during the progression of gastric carcinoma with microsatellite instability. *Lab. Invest.* 79, 1113-1120.
- Kushima R., Muller W., Stolte M. and Borchard F. (1996). Differential p53 protein expression in stomach adenomas of gastric and intestinal phenotypes: possible sequences of p53 alteration in stomach carcinogenesis. *Virchows Arch.* 428, 223-227.
- Lauren P. (1965). The two histological main types of gastric carcinoma; diffuse and so-called intestinal type carcinoma. An attempt at a histochemical classification. *Acta Pathol. Microbiol. Scand.* 64, 31-49.
- Leung W.K., Kim J.J., Kim J.G., Graham D.Y. and Sepulveda A.R. (2000). Microsatellite instability in gastric intestinal metaplasia in patients with and without gastric cancer. *Am. J. Pathol.* 56, 37-43.
- Levine A.J. (1997). p53, the cellular gatekeeper for growth and division. *Cell* 88, 323-331.
- Machado J.C., Soares P., Carneiro F., Rocha A., Beck S., Blin N., Bex G. and Sobrinho-Simoes M. (1999). *E-cadherin* gene mutations provide a genetic basis for the phenotypic divergence of mixed gastric carcinomas. *Lab. Invest.* 79, 459-465.
- Maesawa C., Tamura G., Suzuki Y., Ogasawara S., Sakata K., Kashiwaba M. and Satodate R. (1995). The sequential accumulation of genetic alterations characteristic of the colorectal adenoma-carcinoma sequence does not occur between gastric adenoma and adenocarcinoma. *J. Pathol.* 176, 249-258.
- McKie A.B., Filipe M.I. and Lemoine N.R. (1993). Abnormalities affecting the *APC* and *MCC* tumour suppressor gene loci on chromosome 5q occur frequently in gastric cancer but not in pancreatic cancer. *Int. J. Cancer* 55, 598-603.
- Miyoshi Y., Ando H., Nagase H., Nishisho I., Horii A., Miki Y., Mori T., Utsunomiya J., Baba S., Petersen G., Hamilton S.R., Kinzler K.W., Vogelstein B. and Nakamura Y. (1992a). Germ-line mutations of the *APC* in 53 familial adenomatous polyposis patients. *Proc. Natl. Acad. Sci. USA* 89, 4452-4456.
- Miyoshi Y., Nagase H., Ando H., Horii A., Ichii S., Nakatsuru S., Aoki T., Miki Y., Mori T. and Nakamura Y. (1992b). Somatic mutations of the *APC* gene in colorectal tumors: mutation cluster region in the *APC* gene. *Hum. Mol. Genet.* 1, 229-233.
- Muta H., Noguchi M., Kanai Y., Ochiai A., Nawata H. and Hirohashi S. (1996). *E-cadherin* gene mutation in signet ring cell carcinoma of the stomach. *Jpn. J. Cancer Res.* 87, 843-848.
- Nakamura K., Sakaguchi H. and Enjoji M. (1988). Depressed adenoma of the stomach. *Cancer* 62, 2197-2202.
- Nakamura K., Sugano H. and Takagi K. (1968). Carcinoma of the stomach in incipient phase: its histogenesis and histological appearances. *Gann* 59, 251-258.
- Nakashima H., Honda M., Inoue H., Shibuta K., Arinaga S., Era S., Ueo H., Mori M. and Akiyoshi T. (1995). Microsatellite instability in multiple gastric cancers. *Int. J. Cancer* 64, 239-242.
- Nishizuka S., Tamura G., Maesawa C., Sakata K., Suzuki Y., Iwaya T., Terashima M., Saito K. and Satodate R. (1997a). Analysis of the *DPC4* gene in gastric carcinoma. *Jpn. J. Cancer Res.* 88, 335-339.
- Nishizuka S., Tamura G., Terashima M. and Satodate R. (1997b). Commonly deleted region on the long arm of chromosome 7 in differentiated adenocarcinoma of the stomach. *Br. J. Cancer* 76, 1567-1571.
- Nishizuka S., Tamura G., Terashima M. and Satodate R. (1998). Loss of heterozygosity during development and progression of differentiated adenocarcinoma of the stomach. *J. Pathol.* 185, 38-43.
- Nozawa H., Oda E., Ueda S., Tamura G., Maesawa C., Muto T., Taniguchi T. and Tanaka N. (1998). Functionally inactivating point mutation in the tumor-suppressor *IRF-1* gene identified in human gastric cancer. *Int. J. Cancer* 12, 522-527.
- Ochiai A., Yamauchi Y. and Hirohashi S. (1996). p53 mutations in the non-neoplastic mucosa of the human stomach showing intestinal metaplasia. *Int. J. Cancer* 69, 28-33.
- Ogata S., Tamura G., Endoh Y., Sakata K., Ohmura K. and Motoyama T. (2001). Microsatellite alterations and target gene mutations in the early stage of multiple gastric cancer. *J. Pathol.* 194, 334-340.
- Ohmura K., Tamura G., Endoh Y., Sakata K., Takahashi T. and Motoyama T. (2000). Microsatellite alterations in differentiated-type adenocarcinomas and precancerous lesions of the stomach: with special reference to cellular phenotype. *Hum. Pathol.* 31, 1031-1035.
- Perucho M. (1996). Microsatellite instability: the mutator that mutates the other mutator. *Nat. Med.* 2, 630-631.
- Ranzani G.N., Luinetti O., Padovan L.S., Calistri D., Renault B., Burrell M., Amadori D., Fiocca R. and Solcia E. (1995). p53 gene mutations and protein nuclear accumulation are early events in intestinal type gastric cancer but late events in diffuse type. *Cancer Epidemiol.*

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- Biomarkers Prev. 4, 223-231.
- Rugge M., Correa P., Dixon M.F., Hattori T., Leandro G., Lewin K., Riddell R.H., Sipponen P. and Watanabe H. (2000). Gastric dysplasia: the Padova international classification. *Am. J. Surg. Pathol.* 24, 167-176.
- Saito A., Kanai Y., Maesawa C., Ochiai A., Torii A. and Hirohashi S. (1999). Disruption of E-cadherin-mediated cell adhesion systems in gastric cancers in young patients. *Jpn. J. Cancer Res.* 90, 993-999.
- Sakata K., Tamura G., Maesawa C., Suzuki Y., Terashima M., Satoh K., Eda Y., Suzuki A., Sekiyama S. and Satodate R. (1995). Loss of heterozygosity on the short arm of chromosome 9 without *p16* gene mutation in gastric carcinomas. *Jpn. J. Cancer Res.* 86, 333-335.
- Sakata K., Tamura G., Nishizuka S., Maesawa C., Suzuki Y., Iwaya T., Terashima M., Saito K. and Satodate R. (1997). Commonly deleted regions on the long arm of chromosome 21 in differentiated adenocarcinoma of the stomach. *Genes Chromosom. Cancer* 18, 318-321.
- Sakata K., Tamura G., Ogata S., Ohmura K., Endoh Y. and Motoyama T. (2002). Hypermethylation of *hMLH1* promoter in solitary and multiple gastric cancers with microsatellite instability. *Br. J. Cancer* (in press).
- Sato K., Tamura G., Tsuchiya T., Endoh Y., Usuba O., Kimura W. and Motoyama T. (2001a). Frequent loss of expression without sequence mutations in the *DCC* gene in gastric cancer. *Br. J. Cancer* 85, 199-203.
- Sato K., Tamura G., Tsuchiya T., Endoh Y., Sakata K., Usuba O., Kimura W., Terashima M., Nishizuka S., Zou T., Meltzer S.J. and Motoyama T. (2001b). Analysis of genetic and epigenetic alterations of the *PTEN* gene in gastric cancer. *Virchows Arch.* (in press)
- Schlemper R.J., Itabashi M., Kato Y., Lewin K.J., Riddell R.H., Shimoda T., Sipponen P., Stolte M., Watanabe H., Takahashi H. and Fujita R. (1997). Differences in diagnostic criteria for gastric carcinoma between Japanese and western pathologists. *Lancet* 349, 1725-1729.
- Schmidt P.H., Lee J.R., Joshi V., Playford R.J., Poulson R., Wright N.A. and Goldenring J.R. (1999). Identification of a metaplastic cell lineage associated with human gastric adenocarcinoma. *Lab. Invest.* 79, 639-646.
- Semba S., Yokozaki H., Yasui W. and Tahara E. (1998). Frequent microsatellite instability and loss of heterozygosity in the region including *BRCA1* (17q21) in young patients with gastric cancer. *Int. J. Oncol.* 12, 1245-1251.
- Suzuki H., Itoh F., Toyota M., Kikuchi T., Kakiuchi H., Hinoda Y. and Imai K. (1999). Distinct methylation pattern and microsatellite instability in sporadic gastric cancer. *Int. J. Cancer* 83, 309-313.
- Tahara E. (1995). Genetic alterations in human gastrointestinal cancers: The application to molecular diagnosis. *Cancer* 75S, 1410-1417.
- Takeichi M. (1991). Cadherin cell adhesion receptors as a morphogenetic regulator. *Science* 251, 1451-1455.
- Tamura G., Kihana T., Nomura K., Terada M., Sugimura T. and Hirohashi S. (1991). Detection of *p53* gene mutations in primary gastric cancer by cell sorting and polymerase chain reaction single-strand conformation polymorphism analysis. *Cancer Res.* 51, 3056-3058.
- Tamura G., Maesawa C., Suzuki Y., Ogasawara S., Terashima M., Saito K. and Satodate R. (1993). Primary gastric carcinoma cells frequently lose heterozygosity at the *APC* and *MCC* genetic loci. *Jpn. J. Cancer Res.* 84, 1015-1018.
- Tamura G., Maesawa C., Suzuki Y., Tamada H., Satoh M., Ogasawara S., Kashiwaba M. and Satodate R. (1994). Mutations of the *APC* gene occur during early stages of gastric adenoma development. *Cancer Res.* 54, 1149-1151.
- Tamura G., Sakata K., Maesawa C., Suzuki Y., Terashima M., Satoh K., Sekiyama S., Suzuki A., Eda Y. and Satodate R. (1995). Microsatellite alterations in adenoma and differentiated adenocarcinoma of the stomach. *Cancer Res.* 55, 1933-1936.
- Tamura G., Maesawa C. and Satodate R. (1996a). Author's reply. *J. Pathol.* 178, 476.
- Tamura G. (1996b). Molecular pathogenesis of adenoma and differentiated adenocarcinoma of the stomach. *Pathol. Int.* 46, 834-841.
- Tamura G., Ogasawara S., Nishizuka S., Sakata K., Maesawa C., Suzuki Y., Terashima M., Saito K. and Satodate R. (1996c). Two distinct regions of deletion on the long arm of chromosome 5 in differentiated adenocarcinomas of the stomach. *Cancer Res.* 56, 612-615.
- Tamura G., Sakata K., Nishizuka S., Maesawa C., Suzuki Y., Iwaya T., Terashima M., Saito K. and Satodate R. (1996d). Inactivation of the *E-cadherin* gene in primary gastric carcinomas and gastric carcinoma cell lines. *Jpn. J. Cancer Res.* 87, 1153-1159.
- Tamura G., Sakata K., Nishizuka S., Maesawa C., Suzuki Y., Terashima M., Eda Y. and Satodate R. (1996e). Allelotype of adenoma and differentiated adenocarcinoma of the stomach. *J. Pathol.* 180, 371-377.
- Tamura G., Yin J., Wang S., Fleisher A.S., Zou T.-T., Abraham J.M., Kong D., Smolinski K.N., Wilson K.T., James S.P., Silverberg S.G., Nishizuka S., Terashima M., Motoyama T. and Meltzer S.J. (2000). *E-cadherin* gene promoter hypermethylation in primary human gastric carcinomas. *J. Natl. Cancer Inst.* 92, 569-573.
- Tamura G., Sato K., Akiyama S., Tsuchiya T., Endoh Y., Usuba O., Kimura W., Nishizuka S. and Motoyama T. (2001). Molecular characterization of undifferentiated-type gastric carcinoma. *Lab. Invest.* 81, 593-598.
- Tatematsu M., Hasegawa R., Ogawa K., Kato T., Ichinose M., Miki K. and Ito N. (1992). Histogenesis of human stomach cancers based on assessment of differentiation. *J. Clin. Gastroenterol.* 14 (Suppl. 1), S1-7.
- Tsuchiya T., Tamura G., Sato K., Endoh Y., Sakata K., Jin Z., Motoyama T., Usuba O., Kimura W., Nishizuka S., Wilson K.T., James S.P., Yin J., Fleisher A.S., Zou T., Kong D., Silverberg S.G. and Meltzer S.J. (2000). Distinct methylation patterns of two *APC* gene promoters in normal and cancerous gastric epithelia. *Oncogene* 27, 3642-3646.
- Uchino S., Noguchi M., Ochiai A., Saito T., Kobayashi M. and Hirohashi S. (1993). *p53* mutation in gastric cancer: a genetic model for carcinogenesis is common to gastric and colorectal cancer. *Int. J. Cancer* 54, 759-764.
- Wu M.S., Lee C.W., Shun C.T., Wang H.P., Lee W.J., Chang M.C., Sheu J.C. and Lin J.T. (2000). Distinct clinicopathologic and genetic profiles in sporadic gastric cancer with different mutator phenotypes. *Genes Chromosomes Cancer* 27, 403-411.
- Yamamoto H., Perez-Piteira J., Yoshida T., Terada M., Itoh F., Imai K. and Perucho M. (1999). Gastric cancers of the microsatellite mutator phenotype display characteristic genetic and clinical features. *Gastroenterology* 116, 1348-1357.