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, 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.

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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 G1 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 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[15,55]. 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[56].

**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[57], while early undifferentiated carcinomas show no evidence of MSI (as described below)[57]. *hMLH1* methylation is frequently observed in gastric cancers from elderly patients[58] and has also been described in non-neoplastic gastric epithelia surrounding gastric cancers with MSI[55,57]. 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 plays a role in organogenesis and morphogenesis[59]. Germline *E-cadherin* mutations have been reported in familial diffuse-type of gastric cancers[60]. *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[61,62]. While nearly half of the undifferentiated-scattered (diffuse) type gastric carcinomas contain *E-cadherin* mutations[62,63], such mutations are rare in early undifferentiated carcinomas[56,64], 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[64,65], 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[17,46,34,64]. While *APC* promoter methylation has also been reported in colorectal and other human neoplasms[65], *APC* methylation (promoter 1A) does not appear to be oncopgenic in gastric cancer[18]. Mutation and promoter methylation of *DCC*,...
$p16$, and $PTEN$ genes have also been investigated in gastric cancer$^{[14-23,50,60]}$. 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$^{[14,22,23]}$. DAP-kinase promoter methylation is more frequent in undifferentiated than in differentiated type tumors$^{[31,30,32]}$. While $RASSF1A$ gene mutations are uncommon, silencing of the gene by promoter methylation is frequent in carcinomas, including gastric carcinomas$^{[19,57]}$. 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$^{[20]}$. 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$^{[58]}$.

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$^{[60]}$. 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$^{[20]}$. 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$^{[70-73]}$. 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$^{[2,32]}$. 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$^{[57,58]}$. 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$^{[58]}$, other reports observe the opposite findings$^{[79]}$. Again, these contradictory observations may be due to the frequent conversion of differentiated- to undifferentiated-type tumors$^{[40]}$, 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$^{[77]}$. In contrast, no evidence of MSI has been found in early undifferentiated carcinomas$^{[77]}$. Gastric cancers with an MSI phenotype rarely exhibit structural alterations, such as mutations or LOH of tumor suppressor genes$^{[46,47,60]}$, 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 $SYN$**

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$^{[84]}$. Furthermore, patients exhibiting $MAGE-A1$ and -$A3$ promoter demethylation tend to have a worse prognosis, as assessed by the log rank test$^{[81]}$. Demethylation of both $MAGE-A1$ 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 $SYN$-$\gamma$ ($SNCG$) gene, also known as breast cancer specific gene 1 ($BCNG1$), is a member of the synuclein neuronal protein family, along with $SYN$-$\alpha$ ($SNCA$) and $SYN$-$\beta$ ($SNCB$)$^{[84,86]}$. SNCG protein expression is highly tissue-specific, being expressed at presynaptic terminals in the brain and peripheral nervous system$^{[83,86]}$. 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$^{[84]}$. 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, and only partial demethylation of \(SNCG\) is present in non-neoplastic gastric epithelia. 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. 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, with mutations of \(APC\) gene being the only relatively frequent (20%) DNA structural alteration. Indeed, \(APC\) gene mutations are more frequent in gastric adenomas than in differentiated or undifferentiated gastric carcinomas. More recently, we reported that results of the Padova international classification, correlated with both molecular and cellular phenotypic profiles, and that \(p53\) and hMLH1 immunohistochemistry clearly discriminated these lesions. 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. However, detection of certain genetic alterations, such as \(p53\) mutations, LOH, or MSI, in adenomas may be indicative of malignant transformation. It is noteworthy that gastric-type intramucosal neoplasia, often diagnosed as adenoma or dysplasia, frequently shows a mutator defect.

**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. Although frequent MSI has been reported in intestinal metaplasia, there is little evidence of mismatch repair defects in this tissue. \(Helicobacter pylori\) infection can accelerate the hypermethylation of genes such as \(E\)-cadherin. Hypermethylation of tumor suppressor and tumor-related genes increases with age, and is thought to result in field defects in different organs, although the significance of the hypermethylation appears to be dependent on the genomic region analyzed, 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 \(gamae\)-\(p\) 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.

**REFERENCES**

1. Tamura G, Kihana T, Nomura K, Terada M, Sugimura T, Hiroshiro S. Detection of frequent \(p53\) gene mutations in primary gastric cancer by cell sorting and polymerase chain reaction single-strand conformation polymorphism analysis. *Cancer Res* 1991; 51: 3056-3058
2. Becker KF, Atkinson MJ, Reich U, Becker I, Nekarda H, Fleisher AS. \(E\)-cadherin gene mutations provide clues to diffuse type gastric carcinomas. *Cancer Res* 1994; 54: 3845-3852
3. Tamura G, Katana K, Nishizuka S, Maesawa C, Suzuki Y, Iwaya T, Terashima M, Saito K, Satodate R. Inactivation of the \(E\)-cadherin gene in primary gastric carcinomas and gastric carcinoma cell lines. *Jpn J Cancer Res* 1996; 87: 1153-1159
4. Kim IJ, Kang HC, Shin Y, Park HW, Jang SG, Han SY, Lim SK, Lee MR, Chang HJ, Ku JL, Yang HK, Park JG. A TP53-truncating germline mutation (E287X) in a family with characteristics of both hereditary diffuse gastric cancer and Li-Fraumeni syndrome. *J Hum Genet* 2004; 49: 591-595
5. Guilford P, Hopkins J, Harraway J, McLeod M, McLeod N, Harawira P, Taite H, Scoular R, Miller A, Reeve AE. E-cadherin germline mutations in familial gastric cancer. *Nature* 1998; 392: 402-405
6. Tamura G, Yin J, Wang S, Fleisher AS, Zou T, Abraham JM, Kong D, Smolinski KN, Wilson KT, James SP, Silverberg SG, Nishizuka S, Terashima M, Motoyama T, Meltzer SJ. \(E\)-Cadherin gene promoter hypermethylation in primary human gastric carcinomas. *J Natl Cancer Inst* 2000; 92: 569-573
7. Nishizuka S, Tamura G, Terashima M, Satodate R. Loss of heterozygosity during the development and progression of differentiated adenocarcinoma of the stomach. *J Pathol* 1998; 185: 38-43
8. Akiyama Y, Nakasaki H, Nihei Z, Iwama T, Nomizu T, Utsunomiya J, Yuasa Y. Frequent microsatellite instabilities and analyses of the related genes in familial gastric cancers. *Jpn J Cancer Res* 1996; 87: 595-601
9. Semb S, Yokozaki H, Yasui W, Tahara E. Frequent microsatellite instability and loss of heterozygosity in the region including BRCA1 (17q21) in young patients with gastric cancer. *Int J Oncol* 1998; 12: 1245-1251
10. Tamura G, Sakata K, Maesawa C, Suzuki Y, Terashima M, Satoh K, Sekiyama S, Suzuki A, Edu Y, Satodate R. Microsatellite alterations in adenoma and differentiated adenocarcinoma of the stomach. *Cancer Res* 1995; 55: 1933-1936
11. Fleisher AS, Esteller M, Wang S, Tamura G, Suzuki H, Yin J, Zou TT, Abraham JM, Kong D, Smolinski KN, Shi YQ, Rhyu MG, Powell SM, James SP, Wilson KT, Herman JG, Meltzer SJ. Hypermethylation of the hMLH1 gene promoter in human gastric cancers with microsatellite instability. *Cancer Res* 1999; 59: 1090-1095
12. Kim JJ, Baek MJ, Kim L, Kim NG, Lee YC, Song SY, Noh SH, Kim H. Accumulated frameshift mutations at codons nucleotide repeats during the progression of gastric carcinoma
with microsatellite instability. *Lab Invest* 1999; 79: 1113-1120

13 **Fleisher AS**, Esteller M, Tamura G, Rashid A, Stine OC, Yin J, Zou TT, Abraham JM, Kong D, Nishizuka S, James SP, Wilson KT, Herman JG, Meltzer SJ. Hypermethylation of the hMLH1 gene promoter associated with microsatellite instability in early human gastric neoplasia. *Oncogene* 2001; 20: 329-335

14 **Suzuki H**, Itoh F, Toyota M, Kikuchi T, Kikuchi H, Hinoda Y, Imai K. Distinct methylation pattern and microsatellite instability in sporadic gastric cancer. *Int J Cancer* 1999; 83: 309-313

15 **Toyota M**, Ahuja N, Suzuki H, Itoh F, Ohe-Toyota M, Imai K, Baylin SB, Issa JP. Aberrant methylation in gastric cancer associated with the CpG island methylator phenotype. *Cancer Res* 1999; 59: 5438-5442

16 **Endoh Y**, Tamura G, Ajioka Y, Watanabe H, Motoyama T. Frequent hypermethylation of the hMLH1 gene promoter in differentiated-type tumors of the stomach with the gastric foveolar phenotype. *Am J Pathol* 2000; 157: 717-722

17 **Tamura G**, Sato K, Akiami S, Tsuchiyu T, Endoh Y, Usuba O, Kimura W, Nishizuka S, Motoyama T. Molecular characterization of undifferentiated-type gastric carcinoma. *Lab Invest* 2001; 81: 593-598

18 **Tsukiyama T**, Tamura G, Sato K, Endoh Y, Sakata K, Jin Z, Motoyama T, Usuba O, Kimura W, Nishizuka S, Wilson KT, James SP, Yin J, Fleisher AS, Zou T, Silverberg SG, Kong D, Meltzer SJ. Distinct methylation patterns of two APC gene promoters in normal and cancerous gastric epithelium. *Oncogene* 2000; 19: 3642-3646

19 **Kang GH**, Shim YH, Chae KS, Ryu BG, Chi SG. Frequent epigenetic inactivation of RASSF1A by aberrant promoter hypermethylation in human gastric adenocarcinoma. *Cancer Res* 2001; 61: 7034-7038

20 **Leung WK**, Yu J, Ng EK, To KF, Ma PK, Lee TL, Go MY, Chung SC, Sung JJ. Concurrent hypermethylation of multiple tumor-related genes in gastric carcinoma and adjacent normal tissues. *Cancer* 2001; 91: 2294-2301

21 **Kang GH**, Lee S, Kim WH, Lee HW, Kim JC, Ryu MG, Ro JY. Epstein-barr virus-positive gastric carcinoma demonstrates frequent aberrant methylation of multiple genes and constitutes CpG island methylator phenotype-positive gastric carcinoma. *Am J Pathol* 2002; 160: 787-794

22 **Sato K**, Tamura G, Tsukiyama T, Endoh Y, Usuba O, Kimura W, Motoyama T. Frequent loss of expression without sequence mutations of the DCC gene in primary gastric cancer. *Br J Cancer* 2001; 85: 199-203

23 **Sato K**, Tamura G, Tsukiyama T, Endoh Y, Sakata K, Motoyama T, Usuba O, Kimura W, Terashima M, Nishizuka S, Zou T, Meltzer SJ. Analysis of genetic and epigenetic alterations of the hMLH1 gene promoter is associated with microsatellite instability in colorectal and gastric cancer. *Am J Pathol* 2002; 160: 160-165

24 **Honda T**, Tamura G, Waki T, Kawata S, Nishizuka S, Motoyama T. Promoter hypermethylation of the Chfr gene in neoplastic and non-neoplastic gastric epithelia. *Br J Cancer* 2004; 90: 2013-2016

25 **Obata T**, Toyota M, Satoh A, Sasaki Y, Ogi K, Akino K, Suzuki H, Murai M, Kikuchi T, Nita H, Itoh F, Issa JP, Tohoku T, Imai K. Identification of HRK as a target of epigenetic inactivation in colorectal and gastric cancer. *Clin Cancer Res* 2003; 9: 6410-6418

26 **Kanedo A**, Wakazono K, Tsukamoto T, Watanabe N, Yagi Y, Tatematsu M, Kaminishi M, Sugimura T, Ushijima M. Lysyl oxidase is a tumor suppressor gene inactivated by methylation and loss of heterozygosity in gastric cancers. *Cancer Res* 2004; 64: 6410-6415

27 **Goetz SE**, Vogelstein B, Hamilton SR, Feinberg AP. Hypermethylation of DNA from benign and malignant human colon neoplasms. *Science* 1985; 228: 187-190

28 **Narayan A**, Ji W, Zhang XY, Marrogi A, Graff JR, Baylin SB, Uhrlich M. Hypomethylation of pericentromeric DNA in breast adenocarcinomas. *Int J Cancer* 1998; 77: 833-838

29 **Lin CH**, Hsieh SY, Sheen IS, Lee WC, Chen TC, Shyu WC, Liaw YF. Genome-wide hypomethylation in hepatocellular carcinoma. *Cancer Res* 2001; 61: 4238-4248

30 **Bardelli A**, Suter C, Cheong K, Ku KL, Naegeli H, Hawkins N, Ward R. The relationship between hypermethylation and CpG island methylation in colorectal neoplasia. *Am J Pathol* 2003; 162: 1361-1371

31 **Honda T**, Tamura G, Waki T, Kawata S, Terashima M, Nishizuka S, Motoyama T. Demethylation of MAGE promoters during gastric cancer progression. *Br J Cancer* 2004; 90: 838-843

32 **Yamagawa N**, Tamura G, Honda T, Endoh M, Nishizuka S, Motoyama T. Demethylation of the synuclein gamma gene in primary gastric cancers and gastric cancer cell lines. *Clin Cancer Res* 2004; 10: 2447-2451

33 **Lauren P**, The two histological main types of gastric carcinoma: Diffuse and so-called intestinal-type carcinoma an attempt at a histo-clinical classification. *An attempt at a histo-clinical classification. Acta Pathol Microbiol Scand* 1965; 64: 31-49

34 **Nakashima K**, Sugano H, Takagi K. Carcinoma of the stomach in incipient phase: its histogenesis and histological appearances. *Gann* 1968; 59: 251-258

35 **Jass JR**, Filipe MI. The mucin profiles of normal gastric mucosa, intestinal metaplasia and its variants and gastric carcinoma. *Histochim* 1981; 13: 931-939

36 **Endoh Y**, Sakata K, Tamura G, Ohmura K, Ajisaka Y, Watanabe H, Motoyama T. Cellular phenotypes of differentiated-type adenocarcinomas and precancerous lesions of the stomach are dependent on the genetic pathways. *J Pathol* 2000; 191: 255-263

37 **Ohmura K**, Tamura G, Endoh Y, Sakata K, Takahashi T, Motoyama T. Microsatellite alterations in differentiated-type
adencarcinomas and precancerous lesions of the stomach with special reference to cellular phenotype. *Hum Pathol* 2000; 31: 1031-1035

48 Tatematsu M, Hasegawa R, Ogawa K, Kato T, Ichinose M, Miki K, Ito N. Histogenesis of human stomach cancers based on assessment of differentiation. *J Clin Gastroenterol* 1992; 14 Suppl 1: S1-57

49 Tamura G. Genetic and epigenetic alterations of tumor suppressor and tumor-related genes in gastric cancer. *Histol Histopathol* 2002; 17: 323-329

50 Endoh Y, Tamura G, Watanabe H, Motoyama T. Author’s reply. *J Pathol* 2000; 191: 467-468

51 Levine AJ. p53, the cellular gatekeeper for growth and division. *Cell* 1997; 88: 321-331

52 Jones PA. Laird PW. Cancer epigenetics comes of age. *Nat Genet* 1999; 21: 163-167

53 Uchino S, Noguchi M, Ochiai A, Saito T, Kobayashi M, Hirohashi S. p53 mutation in gastric cancer: a genetic model for carcinogenesis is common to gastric and colorectal cancer. *Int J Cancer* 1993; 54: 759-764

54 Maesawa C, Tamura G, Suzuki Y, Ogasawara S, Sakata K, Kashiwaba M, Satodate R. The sequential accumulation of genetic alterations characteristic of the colorectal adenocarcinoma sequence does not occur between gastric adenoma and adenocarcinoma. *J Pathol* 1995; 176: 249-258

55 Ranzani GN, Luinetti O, Padovan LS, Calistri D, Renault B, Burrel M, Amadori D, Fiocca R, Solcia E. p53 gene mutations and protein nuclear accumulation are early events in intestinal type gastric cancer but late events in diffuse type. *Cancer Epidemiol Biomarkers Prev* 1995; 4: 223-231

56 Herman JG, Umar A, Polya K, Graff JR, Ahuja N, Issa JP, Markowitz S, Willson JK, Hamilton SR, Sidransky D, Preisinger AC. Incidence and functional consequences of hMLH1 promoter hypermethylation in colorectal carcinoma. *Proc Natl Acad Sci U S A* 1998; 95: 6670-6675

57 Guo RJ, Araiz H, Igarashi H, Hemmi H, Araiz H, Hanai H, Sugimura H. Microsatellite instability of papillary subtype of human gastric adenocarcinoma and hMLH1 promoter hypermethylation in the surrounding mucosa. *Pathol Int* 2001; 51: 240-247

58 Nakashima H, Honda M, Inoue H, Shibuta K, Arinaga S, Era S, Ueo H, Morii M, Akiyoshi T. Microsatellite instability in multiple gastric cancers. *Int J Cancer* 1995; 64: 239-242

59 Takeichi M. Cadherin cell adhesion receptors as a tumor suppressor. *Science* 1991; 251: 1451-1455

60 Muta H, Noguchi M, Kanai Y, Ochiai A, Nawata H, Hirohashi S. E-cadherin gene mutations in signet ring cell carcinoma of the stomach. *Jpn J Cancer Res* 1996; 87: 843-848

61 Machado JC, Soares P, Carneiro F, Rocha A, Beck S, Blin N, Berx G, Sobrinho-Simões M. E-cadherin gene mutations provide a genetic basis for the phenotypic divergence of mixed gastric carcinomas. *Lab Invest* 1997; 79: 439-465

62 Miyoshi Y, Ando H, Horii A, Ichii S, Nakatsuru S, Aoki T, Miki Y, Morii T, Utsunomiya J, Baba S, Petersen G. Germ-line mutations of the APC gene in 53 familial adenomatous polyposis patients. *Proc Natl Acad Sci U S A* 1992; 89: 4452-4456

63 Miyoshi Y, Nagase H, Ando H, Horii A, Ichii S, Nakatsuru S, Aoki T, Miki Y, Morii T, Nakamura Y. Somatic mutations of the APC gene in colorectal tumors: mutation cluster region in the APC gene. *Hum Mol Genet* 1992; 1: 229-233

64 Horii A, Nakatsuru S, Miyoshi Y, Ichii S, Nagase H, Kato Y, Yanagisawa A, Nakamura Y. The APC gene, responsible for familial adenomatous polyposis, is mutated in human gastric cancer. *Cancer Res* 1992; 52: 3231-3233

65 Esteller M, Sparks A, Toyota M, Sanchez-Cespedes M, Capella G, Peinado MA, Gonzalez S, Tarafa G, Sidransky D, Meltzer SJ, Baylin SB, Herman JG. Analysis of adenomatous polyposis coli promoter hypermethylation in human cancer. *Cancer Res* 2000; 60: 636-641

66 Sakata K, Tamura G, Maesawa C, Suzuki Y, Terashima M, Satoh K, Eya Y, Suzuki A, Sekiyama S, Satodate R. Loss of heterozygosity on the short arm of chromosome 9 without p16 gene mutation in gastric carcinomas. *Jpn J Cancer Res* 1995; 86: 333-335

67 Dammann R, Schagdarsurengin U, Strunnikova M, Rastetter M, Seidel C, Liu L, Tommasi S, Pfeifer GP. Epigenetic inactivation of the Ras-association domain family 1 (RASSF1A) gene and its function in human carcinogenesis. *Histol Histopathol* 2003; 18: 665-677

68 Homma N, Tamura G, Honda T, Jin Z, Ohmura K, Kawata S, Motoyama T. Hypermethylation of Chf and hMLH1 in gastric noninvasive and early invasive neoplasias. *Virchows Arch* 2005; 446: 120-126

69 Ushijima T. Detection and interpretation of altered methylation patterns in cancer cells. *Nat Rev Cancer* 2005; 5: 223-231

70 Tamura G, Ogasawara S, Nishizuka S, Sakata K, Maesawa C, Suzuki Y, Terashima M, Saito K, Satodate R. Two distinct regions of deletion on the long arm of chromosome 5 in differentiated adenocarcinomas of the stomach. *Cancer Res* 1996; 58: 612-615

71 Tamura G, Sakata K, Nishizuka S, Maesawa C, Suzuki Y, Terashima M, Eya Y, Satodate R. Alleloype of adenoma and differentiated adenocarcinoma of the stomach. *J Pathol* 1996; 180: 371-377

72 Nishizuka S, Tamura G, Terashima M, Satodate R. Commonly deleted region on the long arm of chromosome 7 in differentiated adenocarcinoma of the stomach. *Br J Cancer* 1997; 76: 1567-1571

73 Sakata K, Tamura G, Nishizuka S, Maesawa C, Suzuki Y, Iwaya T, Terashima M, Saito K, Satodate R. Commonly deleted regions on the long arm of chromosome 21 in differentiated adenocarcinoma of the stomach. *Genes Chromosomes Cancer* 1997; 18: 318-321

74 McKie AB, Filipe MJ, Lemoine NR. 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* 1993; 53: 598-603

75 Nozawa H, Oda E, Ueda S, Tamura G, Maesawa C, Muto T, Taniguchi T, Tanaka N. Functionally inactivating point mutation in the tumor-suppressor IRF-1 gene identified in human gastric cancer. *Int J Cancer* 1998; 77: 522-527

76 Nishizuka S, Tamura G, Maesawa C, Sakata K, Suzuki Y, Iwaya T, Terashima M, Saito K, Satodate R. Analysis of the DPC4 gene in gastric carcinoma. *Jpn J Cancer Res* 1997; 88: 335-339

77 Boland CR, Thibodeau SN, Hamilton SR, Sidransky D, Esleman JR, Burt RW, Meltzer SJ, Rodriguez-Bigas MA, Fodde R, Ranzani GN, Srivastava S. 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* 1998; 58: 5248-5257

78 Yamamoto H, Perez-Pistles J, Yoshiida T, Terada M, Itoh F, Imai K, Peruch M. Gastric cancers of the microsatellite mutator phenotype display characteristic genetic and clinical features. *Gastroenterology* 1999; 116: 1348-1357

79 Han HJ, Yanagisawa A, Kato Y, Park JG, Nakamura Y. Genetic instability in pancreatic cancer and poorly differentiated type of gastric cancer. *Cancer Res* 1993; 53: 5087-5089

80 Ogata S, Tamura G, Endoh Y, Sakata K, Ohmura K, Motoyama T. Microsatellite alterations and target gene mutations in the early stages of multiple gastric cancer. *J Pathol* 2001; 194: 334-340

81 Marchand M, van Baren N, Weynants P, Brichard V, Dréno B, Tessler MH, Rankin E, Parmiani G, Arienti F, Humbert Y, Bourdon A, Vanwijck R, Lienard D, Beauduin M, Dietrich PY, Russo V, Kerger J, Masucci G, Jager E, De Greve J, Atzpodien J, Brassee F, Couillé PG, van der Bruggen P, Boon T. Tumor regressions observed in patients with metastatic melanoma treated with an antigenic peptide encoded by gene MAGE-3 and presented by HLA-A1. *Int J Cancer* 1999; 80: 219-230

82 Nishiyama T, Tachibana M, Horiguchi Y, Nakamura K, Ikeda Y, Takesako K, Murai M. Immunotherapy of bladder cancer using autologous dendritic cells pulsed with human lymphocytic antigen-A24-specific MAGE-3 peptide. *Clin Cancer Res* 2001; 7: 25-31

83 Sadanaga N, Nagashima H, Mashino K, Tahara K, Yamaguchi
H, Ohta M, Fujie T, Tanaka F, Inoue H, Takesako K, Akiyoshi T, Mori M. Dendritic cell vaccination with MAGE peptide is a novel therapeutic approach for gastrointestinal carcinomas. *Clin Cancer Res* 2001; 7: 2277-2284

84 Jakes R, Spillantini MG, Goedert M. Identification of two distinct synucleins from human brain. *FEBS Lett* 1994; 345: 27-32

85 Lavedan C, Leroy E, Dehejia A, Buchholtz S, Dutra A, Nussbaum RL, Polymeropoulos MH. Identification, localization and characterization of the human gamma-synuclein gene. *Hum Genet* 1998; 103: 106-112

86 Ninkina NN, Alimova-Kost MV, Paterson JW, Delaney L, Cohen BB, Imreh S, Gnuchev NV, Davies AM, Buchman VL. Organization, expression and polymorphism of the human persyn gene. *Hum Mol Genet* 1998; 7: 1417-1424

87 Gupta A, Godwin AK, Vandermeer L, Lu A, Liu J. Hypomethylation of the synuclein gamma gene CpG island promotes its aberrant expression in breast carcinoma and ovarian carcinoma. *Cancer Res* 2003; 63: 664-673

88 Schlemper RJ, Itabashi M, Kato Y, Lewin KJ, Riddell RH, Shimoda T, Sipponen P, Stolte M, Watanabe H, Takahashi H, Fujita R. Differences in diagnostic criteria for gastric carcinoma between Japanese and western pathologists. *Lancet* 1997; 349: 1725-1729

89 Rugge M, Correa P, Dixon MF, Hattori T, Leandro G, Lewin K, Riddell RH, Sipponen P, Stolte M, Watanabe H. Gastric dysplasia: the Padova international classification. *Am J Surg Pathol* 2000; 24: 167-176

90 Tamura G, Maesawa C, Satodate R. Author’s reply. *J Pathol* 1996; 178: 476

91 Tamura G, Maesawa C, Suzuki Y, Tamada H, Satoh M, Ogasawara S, Kashiwaba M, Satodate R. Mutations of the APC gene occur during early stages of gastric adenoma development. *Cancer Res* 1994; 54: 1149-1151

92 Jin Z, Tamura G, Honda T, Motoyama T. Molecular and cellular phenotypic profiles of gastric noninvasive neoplasia. *Lab Invest* 2002, 82: 1637-1645

93 Nakamura K, Sakaguchi H, Enjoji M. Depressed adenoma of the stomach. *Cancer* 1988; 62: 2197-2202

94 Tamura G. Molecular pathogenesis of adenoma and differentiated adenocarcinoma of the stomach. *Pathol Int* 1996; 46: 834-841

95 Kushima R, Müller W, Stolte M, Borchard F. Differential p53 protein expression in stomach adenomas of gastric and intestinal phenotypes: possible sequences of p53 alteration in stomach carcinogenesis. *Virchows Arch* 1996; 428: 223-227

96 Ochiai A, Yamauchi Y, Hirohashi S. p53 mutations in the non-neoplastic mucosa of the human stomach showing intestinal metaplasia. *Int J Cancer* 1996; 69: 28-33

97 Leung WK, Kim JJ, Kim JG, Graham DY, Sepulveda AR. Microsatellite instability in gastric intestinal metaplasia in patients with and without gastric cancer. *Am J Pathol* 2000; 156: 537-543

98 Jin Z, Tamura G, Satoh M, Meguro T, Miura T, Hayashi M, Osakabe M, Ohmura K, Ogata S, Endoh Y, Motoyama T. Absence of BAT-26 instability in gastric intestinal metaplasia. *Pathol Int* 2001; 51: 473-475

99 Chan AO, Lam SK, Wong BC, Kwong YL, Rashid A. Gene methylation in non-neoplastic mucosa of gastric cancer: age or *Helicobacter pylori* related? *Am J Pathol* 2003; 163: 370-371; author reply 370-371

100 Waki T, Tamura G, Sato M, Motoyama T. Age-related methylation of tumor suppressor and tumor-related genes: an analysis of autopsy samples. * Oncogene* 2003; 22: 4128-4133

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