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■ REVIEW ■

Genetic Events Underlying Morphological Complexity of Gastric Carcinoma

Cancer is a genetic disorder in which gene alterations are selected to provide growth advantage by oncogene activation and/or tumor suppressor gene inactivation. Even marked intra-tumor variation in the histologic pattern, which is common in gastric carcinoma, is considered a result of distinct oncogenic pathways coexisting together. The present review describes that most gastric carcinomas arise through two distinct genetic pathways: microsatellite instability targeting the mononucleotide tracts within coding regions of cancer-related genes and chromosomal deletion involving tumor suppressor genes. With regard to malignant phenotypes, microsatellite instability is associated with the intestinal histological type and chromosomal deletion is correlated with the growth pattern of gastric carcinoma. Moreover, the genetic instability would in turn lead to an increase in alterations of cancer-related genes. The corresponding cells gradually manifest diverse neoplastic properties, thus bringing about consecutive subclonal evolution of more malignant cells. We now have some clues leading to the characterization of phenotypic complexity of gastric carcinoma based on gene-inactivation mechanisms.

Key Words : *Gastric carcinoma, Chromosomal deletion, Genetic instability, Histological type, Growth pattern*

Mun-Gan Rhyu

Department of Microbiology, College of Medicine, The Catholic University of Korea

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Address for correspondence
Mun-Gan Rhyu, M.D.
Department of Microbiology, College of Medicine, The Catholic University of Korea, 505 Banpo-dong, Socho-gu, Seoul 137-701, Korea
Tel : +82.2-590-1215, Fax : +82.2-596-8969
E-mail : rhyumung@cmc.cuk.ac.kr

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CANCER IS A GENETIC DISORDER

Decades of research have built up a variety of evidence proving that cancer is a genetic disorder advanced by alterations on tumor cell genomes accumulating. Genetic alterations would be selected to provide growth advantage by oncogene activation and/or tumor suppressor gene inactivation (1). Activated oncogenes are identified in transformed cells but are rarely or never inherited as a constitutional mutation. On the other hand, many hereditary cancers demonstrate inactivation of tumor suppressor genes via a two-hit mechanism, a germ-line mutation in one allele and a subsequent somatic alteration in the other allele (2, 3). These two distinct alterations in oncogenes and tumor suppressor genes have opened a way to the understanding of mechanisms underlying a tumorigenic multistep process. Loss of tumor suppressor by two rate-limiting genetic hits in particular shows various aspects of genetic progression of both sporadic and hereditary cancers.

The majority of genetic hits in sporadic cancers is an alteration either in DNA sequences of tumor suppressor genes or in multilocus chromosomal events encompassing tumor suppressor genes such as chromosomal deletion (2). According to a genetic model for colorectal tumorigenesis proposed by Fearon and Vogelstein (4), such genetic alterations occur sequentially in at least four to five genes or in a set of normal genes expressed in particular cells during the formation of a malignant tumor. In addition, a mutator phenotype predisposing to gastrointestinal tumors provide intriguing opportunities to unravel consecutive multiple steps involved in malignant progression (5, 6). A number of genetic evidences accumulated to indicate that further interpretation should be given as an addition to clinical diagnosis and cancer treatment which rely largely on its microscopic appearance.

Gastric carcinomas exhibit such a variety of appearances that many microscopic classifications have been made to correlate the histological patterns to survival and mortality rates. The two histopathological types of gastric adenocarcinoma, intestinal and diffuse (7), have been well-known to reflect different histogenetic origins and biological behavior or to predict the clinical outcome. In terms of growth pattern, gastric tumors are subdivided

into two groups, expanding and infiltrative patterns. The growth pattern has also been considered as a helpful guide in predicting overall survival rate (8). The great explosion of findings on genetic events occurring in gastric carcinogenesis makes it likely to bridge genetic alterations and their resultant microscopic appearances. Even marked intra-tumor variation in the histological pattern was considered to reflect the coexistence of distinct mechanisms for gene inactivation or different oncogenic pathways. The present review aims to describe genetic events underlying morphological complexity of gastric carcinoma.

MICRODISSECTION OF TUMOR TISSUE THAT IS GENETICALLY OR PHENOTYPICALLY HOMOGENOUS

Loss of chromosomal region in a solid tumor is studied by loss of heterzygosity (LOH) or allelotype (analysis for genome-wide allelic loss) using highly polymorphic microsatellite markers representing all autosomal chromosome arms. This analysis has two major limitations obscuring the detection of LOH, especially in gastric carcinomas. Genetic alterations of tumor cells are found by comparing tumor DNA with the corresponding normal DNA from the same patient. Gastric tumor specimens are often contaminated with stromal cells and inflammatory cells so that LOH in tumor cells may be undetectable or underestimated due to the high fraction of non-neoplastic DNA. Another limitation comes from the fact that even DNA extracted from tissue with highly pure tumor cells may obscure the presence of intratumoral genetic heterogeneity in homogenised tumor DNA samples (9, 10). Indeed, because gastric carcinomas have marked the heterogeneous intratumoral morphologic variation, multiple tumor sites are needed to be individually examined for genetic alterations according to microscopic differences.

Microdissection is widely used to obtain tissue samples enriched in neoplastic cells showing the same morphologic characteristics. In this approach, adjacent tumor and normal tissues were obtained as a pair from formalin-fixed paraffin-embedded surgical sections (11). To purify tumor cells from normal stromal cells and inflammatory cells, tumor cell-rich tissue areas were selected microscopically and were scraped off using hematoxylin/eosin-stained sections as a reference. This microdissection procedure produces >60% purity of tumor cell populations. Phenotypically homogenous tumor cells can be selected by crypt isolation-based subpopulation (CIBS), which is well-designed to distinguish even intratumoral histological differences (12, 13). Flow sorting based on nuclear DNA content is used to obtain genetically homogenous fraction of tumor cells from surgical or endoscopic biopsy specimens as well as from paraffin-embedded specimens (14-16). Tumor tissue DNA extracted by microdissection or flow-sorting is useful in studying the genetic events underlying heterogenous malignant phenotypes.

TWO DISTINCT MECHANISMS OF GENETIC CHANGES, MICROSATELLITE INSTABILITY AND CHROMOSOMAL DELETION

Genetic instability predisposing to cancer

Microsatellites which are tandem repeats of simple mono-, di-, tri-, and tetranucleotide are dispersed throughout the human genome. Microsatellite DNA is sequentially unstable, thus giving rise to mismatched base pairs during DNA replication. When mismatch repair (MMR) mechanism is impaired, heteroduplex DNA remains uncorrected and the loss or gain of repeat units occurs after a subsequent replication (17). In fact, frequent mutations in short tandem repeat sequences i.e. microsatellite instability (MSI), have been well described as a mutator phenotype caused by MMR defects (6). These microsatellite sequences, therefore, can be a sensitive indicator of genetic instability underlying some tumors (Fig. 1A).

Initially, hypermutability in microsatellite DNA has been observed in hereditary nonpolyposis colorectal cancer (HNPCC; 18, 19), succeeding in a number of other cancers such as sporadic colon (20, 21), gastric (22, 23), pancreatic (24), endometrial (25), esophageal (26) and small cell lung carcinomas (27), and squamous cell carcinoma of the head and neck (28). Based on the genetic linkage analysis, MSI-related genes were mapped to chromosomes 2p (*hMSH2*), 3p (*hMLH1*), 2q (*hPMS1*) and 7p (*hPMS2*) (29-31). Accordingly, HNPCC patients were found to have germ-line mutations in these MMR genes (31-34) indicating that the high frequency of mutations in human cancers are caused by defects in such DNA MMR genes. Among the four MMR genes, germline defects in *hMSH2* and *hMLH1* account for more than 80% of the HNPCC, whereas mutations in *hPMS2* appear to be much more rare (35).

Mouse models for HNPCC genes have given important insights into genomic instability predisposing to cancer. A significant fraction of Msh2-deficient mouse develops lymphoma accompanying MSI-mutator phenotype at an early stage (36, 37). In mice with mutations in *Mlh1* and *Pms2* genes, similar somatic phenotypes such as lymphomas or sarcomas with MSI were frequently observed despite of distinct differences in the meiotic progression (38-40). Furthermore, it has been demonstrated that a mismatch protein of hMSH2 restored mismatch repair to

nuclear extracts of hMSH2-deficient colorectal cells (41, 42). Inactivation of MMR function thus appears to be crucial in increasing the mutation rate and accelerating tumor progression. However, in HNPCC patients, tumorigenesis is mainly restricted to the proximal colon, distal stomach and endometrium. A discrepancy in tissue specificity between HNPCC and the mouse models still awaits further studies.

Widespread and low-level microsatellite instabilities

In addition to gastrointestinal tumors, there have been several studies demonstrating the presence of MSI in esophagus (26), cervix (43), prostate (44), and head and neck (28) cancers using a number of microsatellite markers (20 to 139 markers). Overall, MSI in each tumor varied in the number of mutated markers and in the degree of alteration in length and direction, i.e. the size of inserted or deleted repeat units. Considering the number of mutated markers per each tumor, a considerable number of cancer patients with MSI revealed less frequent mutations than widespread MSI observed in gastrointestinal tumors. These results suggest at least two types of microsatellite mutations, low and high levels. Low MSI below 2.5% may be regarded with a background rate (27). This infrequent alteration is suspected to be the inherent instability of microsatellite sequences which are unstable due to spontaneous DNA strand slippage. A relatively high rate or more than 3% was defined as a distinct mutator phenotype (27, 45).

High or widespread MSI shows frequent mutations in most microsatellite markers tested, representing MMR insufficiency (45). Several attempts including a multicenter study were made to form reproducible MSI criteria and achieve diagnostic sensitivity that we clear the distinction between MSI and nonspecific alterations (46-48). According to the MSI analysis, a uniform panel (number and type) of microsatellite markers should be defined for comparable data between studies because different types of marker repeat have different susceptibility to the mutator phenotype. Those studies using different microsatellite panel have suggested different cutoff values for the percentage of unstable markers that verify high MSI, being varying from 20% to 40%. However, there were few cases of the mutation frequency spanning the borderline cutoff ranges, indicating a bimodal distribution composed of only two fractions, low and high MSI. Therefore, it is reasonable that MSI is subdivided into high MSI (>40% unstable markers) and low MSI (<10% unstable markers) using a uniform panel of microsatellite markers.

High MSI, accompanying frameshift mutations in cancer-related genes, is a distinctive feature observed only in approximately 15-25% of gastrointestinal tumors, (49, 50). Gastrointestinal cancer with high MSI-mutator phenotype specifically illustrates collaborative roles of cancer susceptibility genes and cancer-related genes in tumorigenesis. The genetic instability raised by defects in the MMR genes which are cancer susceptibility genes increases the probability of occurrence of frameshift mutations in cancer-related genes directly responsible for controlling normal cell growth and differentiation. Indeed, the MSI-mutator phenotype has been found to produce frameshift mutations in coding microsatellite sequences of cancer-related genes including *transforming growth factor β receptor type II* (*TGF-β RII*; 51), the *insulin-like growth factor II receptor* (*IGFIIR*; 52), and *BAX* (53).

Genetic progression of a mutator phenotype

In addition to the cancer-related genes, Malkhosyan et al. (54) have found frameshift mutations of *hMSH6* and *hMSH3* genes in about 30% and 40% of mutator phenotype-colorectal cancers, respectively. These frameshift mutations occur within microsatellite-like sequences, a run of $(A)_8$ in the coding region of the *hMSH3* gene and a run of $(C)_8$ in the *hMSH6* gene. Moreover, frameshift mutations in cancer-related genes such as *TGF-β RII* were frequently observed in most MSI-tumor areas from a given patient regardless of the presence of frameshift mutations in the MMR genes, but not vice versa (55). These findings indicate that both *hMSH3* and *hMSH6* frameshift mutations result from previous defective mismatch repair i.e. secondary mutators. Because the two genes in yeast have been known to compose a single MSH2-dependent repair pathway (56), the mutated mutators would subsequently exaggerate the instability phenotype.

Altogether, Perucho (57) proposed a model showing that the MSI-mutator phenotype unfolds in gradual steps by the successive action of different mutator genes. This mutator phenotype pathway consists of at least two primary mutators, *hMSH2* and *hMLH1*, and two secondary mutators, *hMSH3* and *hMSH6*. During tumorigenesis, the activation of primary mutators produce the genetic instability, especially on simple repeated sequences, which targets secondary mutator genes. We further confirmed the genetic progression of gastric cancers with MSI by ordering the frameshift mutations (55). Multiple tumor sites obtained from the same patient were compared to study topographical distribution of MSI-associated frameshift mutations. Initial alterations would be present in all MSI-tumor site, whereas late additional events are restricted to particular MSI-tumor areas. Based on the topographical analysis of MSI-phenotype, *TGF-β RII* and *BAX* were found to be the targets for primary mutator genes. Otherwise, *IGFIIR* mutations were confined within the tumor sites that contained *hMSH3* mutations, indicating the

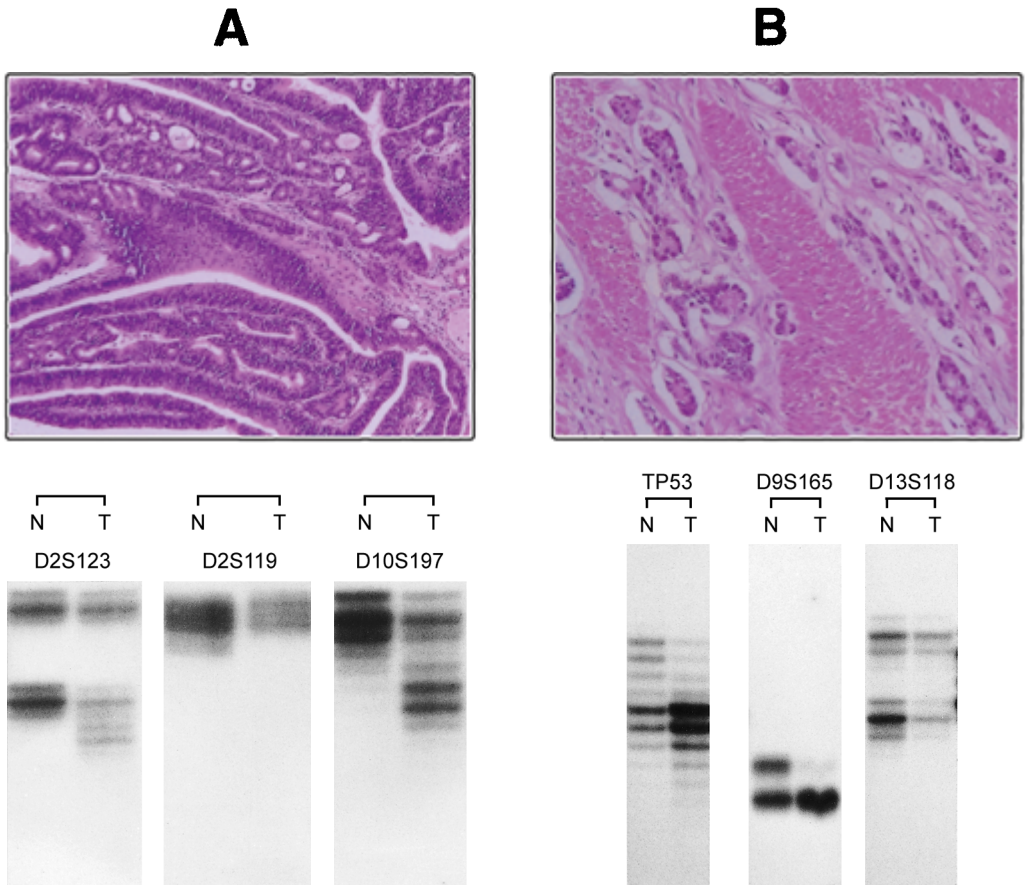
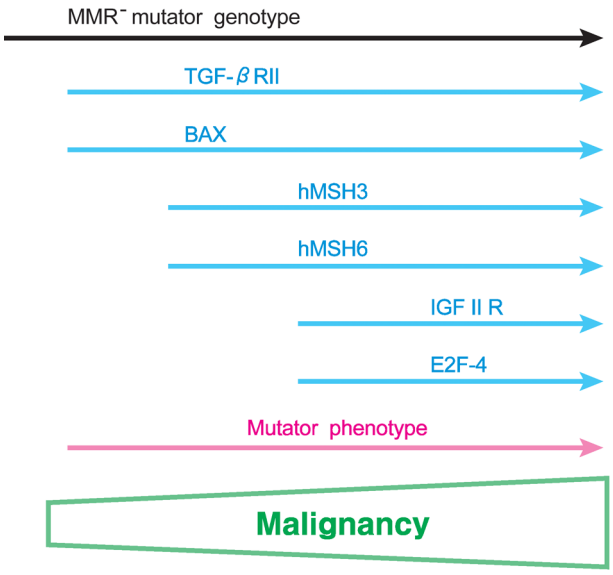


Fig. 1. MSI-associated intestinal-type histology (A) and multiple LOH-induced infiltrative growth pattern (B) of gastric carcinoma (H&E). The microsatellite markers in matched normal (N) tumor (T) DNAs were analyzed by PCR for the presence of allelic gain or loss. A, intestinal-type tumor shows novel alleles that are absent normal tissue at multiple markers, *D2S123, D2S119*, and *D10S197*. Most intestinal-type tumors harbor *TGF-βRII* frameshift mutation, which is thought to play a direct role in the acquisition of malignant phenotype. B, the infiltrative type tumor lost one of two alleles on three chromosome arms, 9p (*9S165*), 13q (*13S118*), and 17p (*TP53*). High level of fractional allelic loss, concurrent DNA loss on various chromosomal regions, appears to be effective on growth potential or power of penetration.



secondary target for the secondary mutator gene. *E2F-4* genes encoding a transcriptional activator is another possible target for the secondary mutator because it has been found to be closely correlated with *hMSH3* mutations (58). Therefore, the mutator phenotype cascade is composed of the two primary and two secondary mutator genes,

Fig. 2. The mutator phenotype cascade composed of primary and secondary mutators. MMR-mutator genotype is initiated as a consequence of defective mismatch repair genes such as *hMSH* and *hMLH* genes. The level of instability phenotype become exaggerated by MSI-associated mutation in secondary mutators such as *hMSH3* and *hMSH6*. These consecutive events exaggerate the instability phenotype, thus broadening the spectrum of cancer-related genes targeted. Resultant mutations in cancer-related genes, *TGF-βRII*, *IGFIIR*, *BAX*, and *E2F-4* lead to clonal expansion of more malignant cells and then manifest diverse neoplastic properties.

accelerating the level of genomic instability. During tumor progression, accelerated mutational inactivation would in turn increase the number of affected genes (Fig. 2). Therefore, it is likely that subclonal tumor cells manifest diverse neoplastic properties and then bring about successive expansions of more malignant cells.

Clinicopathological features of mutator phenotype tumors

Mutator phenotype tumors exhibit clinicopathological characteristics distinguished from conventional tumor suppressor pathways. For instance, alterations of tumor suppressor genes are usually parallel with cytogenetical aberrations leading to aneuploid and chromosomal deletion and/or rearrangement. In contrast, mutator pehnotype tumors maintain diploid or near-diploid status with infrequent LOH (20, 59-61). They reveal a tissue specificity for the distal stomach and proximal colon (21, 61). Moreover, there was a close correlation between a MSI-mutator phenotype and the intestinal-type of gastric carcinomas (61, 62), indicating cell-type specificity for intestinal epithelium (Fig. 1A). Subsequent analyses demonstrated that of MSI-associated mutations, the *TGF-βRII* mutation was most frequently shown in MSI-positive cancers of colon and stomach (49). Our recent study of gastric cancer revealed that the frameshift mutation preferentially associated with the intestinal type of gastric carcinomas (50). TGF-β family, ligand of *TGF-βRII*, is abundant in intestinal mucosa. This family inhibits the growth of epithelial cells and mediates cell differentiation and apoptosis within colon tissue (63-66). In case of intestinal-type carcinoma, it is likely that metaplastic glands in which gastric mucosa bears resemblance to intestinal epithelium become susceptible to the *TGF-βRII* mutation. Thus, the *TGF-βRII* mutation appears to account for why colon and intestinal metaplasia are susceptible to the MSI-mutator phenotype. Inactivating mutations in the *TGF-βRII* gene have been observed in some stomach and colon cancer cells which are resistant to the growth inhibitory activity of TGF-β (51, 67). Furthermore, the TGF-β resistant cell line became sensitive to the growth factor when expressing wild-type TGF-β receptors (68). These findings suggest that the receptor functions as a tumor suppressor in the colon and stomach.

Proximal and distal gastric tumors have been known to be heterogenous entities characterized by distinct epidemiological and biological features. In general, intestinal-type gastric carcinomas are located in the distal stomach with the formation of glandular structures like colonic carcinoma, while the diffuse types invade the stomach, especially the cardia, without forming well-defined structures (7). Analyses from clinical trials have revealed that patients with intestinal-type cancer often show better survival rate than those with diffuse-type (69). The prevalence of intestinal metaplasia is parallel to the high incidence of *Helicobacter pylori* infection and intestinal-type cancer (70, 71). The epidemiological correlation between intestinal metaplasia and *H. pylori* infection supports a postulation that as *H. pylori* infection increases oxidative DNA damage in gastric mucosa, the accumulation of DNA damage transforms normal stomach tissue into intestinal metaplasia (72, 73). These findings imply that intestinal-type gastric carcinoma with MSI might be derived from intestinal metaplasia via *H. pylori* infection-induced DNA damage. Therefore, through *TGF-βRII* mutation the MSI-mutator phenotype is thought to further transform intestinal metaplasia into intestinal-type gastric carcinoma in high-risk populations (Fig. 3). In contrast, widespread MSI occurs infrequently in gastric cardia (74), supporting that there is cell-type specificity of MSI for intestinal cells which are located in the distal stomach as well as in the proximal colon.

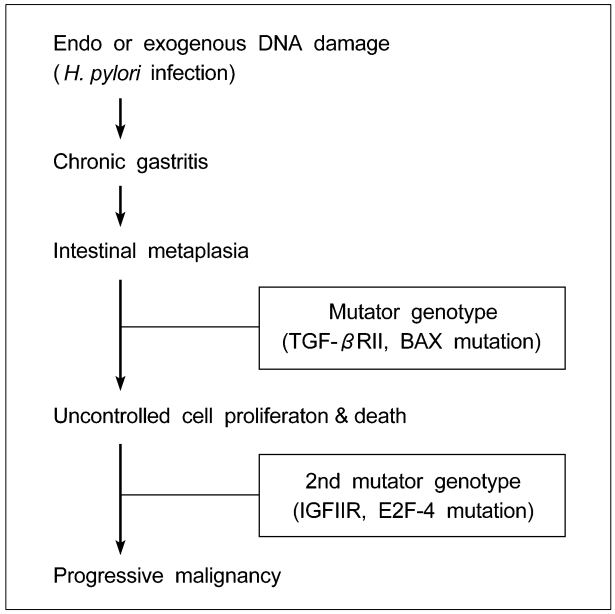


Fig. 3. Genetic pathway of intestinal-type gastric carcinoma with the mutator phenotype.

Chromosomal deletion

Of the various genetic changes causing loss of genetic information, chromosomal (micro)deletion is the most frequent event observed in solid tumor cell genomes (75). Partial loss of a chromosome results from double-strand breaks (DSBs) triggered by ionizing radiation or other DNA damage agents or by endogenous carcinogen produced during normal DNA metabolic process (76). Chromosomal DSBs are repaired by several types of chromosomal recombination, such as homologous and non-

homologous recombinational repairs. Depending on the type of recombination, different chromosomal abnormalities can result. For example, rejoining broken DNA ends may produce intrachromosomal nonhomologous recombination. Occasionally, this type of recombination gives rise to interstitial deletion affecting the variable extent of the chromosome arm of solid tumor cells (77). When such multilocus chromosomal deletion involves tumor suppressor genes, normal cells would be transformed into neoplastic cells by acquiring selective growth advantage and/or immortality (Fig. 1B). Although the end-joining mechanism is commonly responsible for loss of genetic material, LOH events observed in solid tumor tissues do not always represent DNA loss.

A fluorescence in situ hybridization technique has shown that LOH may be either due to chromosomal loss resulting in retention of only one allele or due to gene conversion leading to two homologous alleles (78). There is a difference between the extent of LOH resulting from chromosomal loss and resulting from gene conversion. Using flanking polymorphic markers, the example of chromosomal loss showed concordant LOH encompassing multiple genetic loci in a row. In contrast, non-reciprocal gene conversion resulting in homologous two alleles involves only a single independent marker on chromosome (78, 79). To be certain whether LOH is an outcome of DNA loss or conversion to homologous alleles, flanking microsatellite markers on the corresponding chromosome are necessary to determine the extent of the affected region; deletion involving tumor suppressor gene(s) could manifest LOH at multiple markers, spanning the multilocus chromosomal region. In addition, although high levels of LOH occur on cancer gene-associated chromosomes, non-selected LOH are also seen throughout the cancer genome in the background frequency (<20-30%). LOH on arbitrary chromosomes and conversion to homologous alleles on specific chromosomes could both compromise the correlation between a malignant phenotype and its causative chromosomal deletion. Therefore, a more defined criteria for the loss of genetic material, i.e. consecutive LOH at flanking markers encompassing tumor suppressor genes, is necessary for more accurate correlation between the genotype and phenotype.

Alternatively, tumor and normal tissue make a difference in band intensities, but not big enough to meet criteria stated for defining LOH. The borderline level of LOH or incomplete LOH may be due to heterogenous subpopulations composed of two distinct clones with and without LOH (80). A more precise microdissection such as crypt isolation-based subpopulation analysis is useful to obtain monoclonal genetic information from heterogenous tumor cell populations (13). Allelic imbalance is also caused by allelic gain, which is suspected to be inherent alterations of microsatellite sequences. Because DNA strand slippage constitutionally occurs on simple repeat sequences that are sequentially unstable, infrequent mutations are defined as the background rate of approximately 2.5%. Sometimes, this low-level instability may be indistinguishable from DNA loss. However, allelic gain is thought to make a marginal change in LOH analysis because of its rare occurrence.

Allelotype and fractional allelic loss

Although LOH studies have provided useful information for the construction of chromosomal maps on a number of tumor suppressor genes, there still is disagreement in the role of each tumor suppressor during tumorigenesis. For example, Tahara et al. (81) reported that chromosome 1q, 5q, and 18q were frequently lost in well-differentiated cancers but rarely in poorly differentiated types. On the other hand, LOH on 5q (*APC*), 18q (*DCC*), and 13q (*RB*) was not correlated with the grade of differentiation histology (82). Because LOH implicates loss of genetic material in only one allele, the other allele is necessarily affected by other gene alterations such as mutation for the loss of gene function. Thus, LOH event occurring in a distinct chromosomal region would not be correlated on its own with a malignant feature. However, it is noteworthy that many cancers have their own allelotype obtained by genome-wide LOH analysis. Moreover, individual patients of a given cancer exhibit diverse fractional allelic loss (FAL, the ratio of LOH-positive markers to the total number of informative markers), the overall extent of chromosomes undergoing LOH.

Carrying a genome-wide LOH study, we found multiple nonrandom deletions on chromosomes 17p, 18q, 13q, and 9p in gastric carcinoma, all of which were well-known chromosome arms for displaying frequent allelic loss. A graphic representation of the resulting allelotype is shown in Fig. 4. The allelotype of gastric carcinoma is similar to those of colorectal and esophageal cancers. For instance, colorectal cancers have demonstrated nonrandom LOH on four cancer-associated chromosomes, 5q, 8p, 17p, and 18q (4). Esophageal cancers contained nonrandom LOH on 5q, 9p, 13q, and 17p (83). The similarities in allelotypes of these three gastrointestinal tumors suggest the presence of a common genetic pathway for the development of tumors.

In general, most patients who have multiple nonrandom LOH on the four cancer-associated chromosomes exhibit high levels of FAL. However, FAL values vary from patient to patient, showing a bimodal distribution (84, 85). A genome-wide LOH study on gastric carcinomas using a number of microsatellite markers thus led

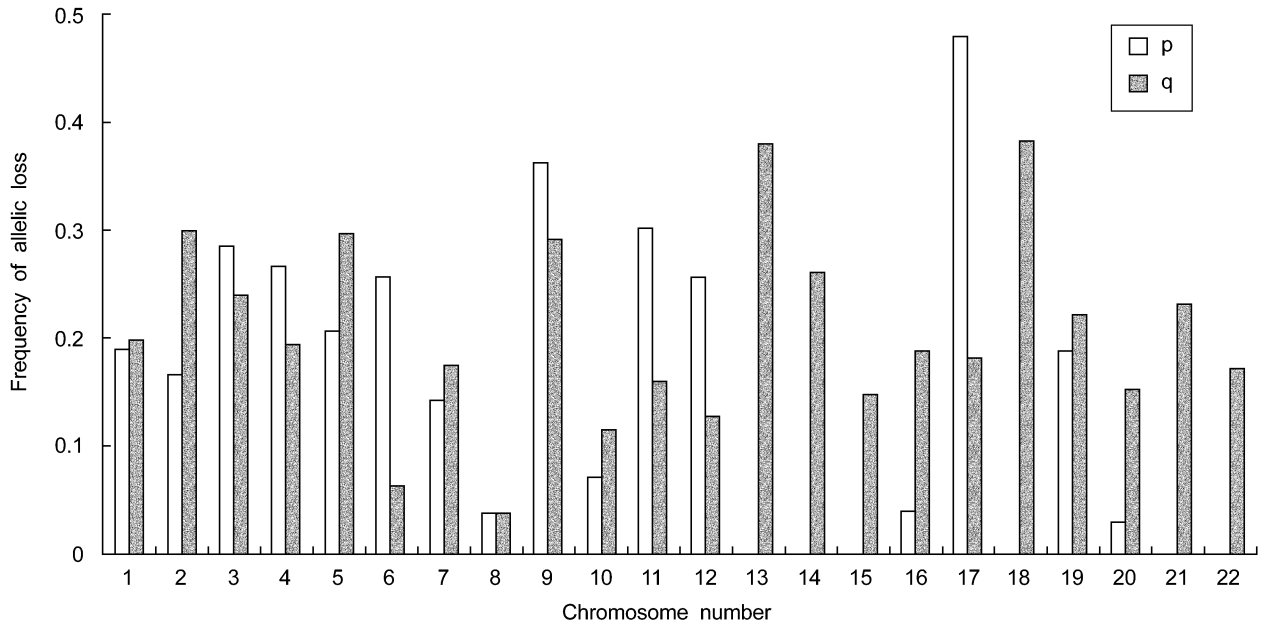


Fig. 4. A graphic presentation of allelotype of gastric carcinoma.

us to subdivide the samples into LOH-related (>mean value) and LOH-unrelated (<mean value) types (86). In LOH-related types, there was a phenotypic implication of FAL value with an increase in the infiltrative type of growth pattern (Fig. 1B). In LOH-unrelated types, because the major tumorigenecity is more likely imposed upon by alterations other than LOH event, no malignant phenotypes correlate with FAL values. The growth pattern of gastric carcinoma is characterized by distinctive microscopic features and by gross appearance of the tumor (8). The cells of expanding carcinoma aggregate and produce a circumscribed mass with limited penetration, whereas the cells of infiltrative carcinoma spread peripherally without forming a tumor mass. Considering the correlation between the overall extent of chromosomal deletion and the growth pattern, concurrent DNA loss on various chromosomal regions appears to influence growth potential or the power of penetration.

MSI AND LOH EVENTS UNDERLIE THE MORPHOLOGICAL COMPLEXITY OF GASTRIC CARCINOMA

Interestingly, there is no correlation between the growth pattern and the MSI mutator phenotype, or between the histological type and chromosomal deletion. This inverse correlation suggests that the presence of premalignant lesion preceding malignant neoplasia or the time at which the gene is affected is more important in determining the histologic type or the degree of differentiation. If intestinal metaplasia of stomach suffers oncogenic frameshift mutations, the lesion could develop into intestinal type gastric carcinoma in one of the two growth patterns. Alternatively, when multiple chromosomal deletions involve gastric mucosa, it could result in infiltrative tumor, irrespective of histological differentiation. However, we found neither LOH nor MSI in 20% of gastric carcinomas. The study on epigenetic alterations such as DNA methylation and genomic imprinting will be helpful to further support this idea about genetic events underlying growth pattern and histology.

Taken together, it is likely that most gastric carcinomas arise through two distinct genetic pathways: chromosomal deletion involving tumor suppressor genes and frameshift mutation targeting microsatellite sequences within coding regions of cancer-related genes (Table 1).

Table 1. Characteristics of two distinct genetic pathways for gastric carcinoma

Features	Mismatch repair deficiency	Chromosomal misrepair
Microsatellite alteration	Microsatellite instability	Loss of heterozygosity
Oncogenic alteration	Frameshift mutation	Fractional allelic loss
Related malignant phenotype	Intestinal type histology	Infiltrative growth pattern

The number of nonrandom chromosomal deletions is correlated with the growth pattern of gastric carcinoma and MSI is associated with the intestinal histological type. However, there is an inverse correlation between MSI and growth patterns, and between LOH and histological types. These findings suggest that the phenotypic complexity of gastric carcinoma is linked to various gene alteration combinations and the mechanism of genetic inactivation. LOH and MSI events are expected to provide important clues to the morphologic complexity of gastric carcinoma and for diagnostic classification on the basis of genetic alteration.

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