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• GASTRIC CANCER •

Mitochondrial microsatellite instability in gastric cancer and its precancerous lesions

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Abstract

AIM: To evaluate the role of mitochondrial microsatellite instability (mtMSI) in gastric carcinogenesis.

METHODS: MtMSI was measured with PCR-single strand conformation polymophism (PCR-SSCP) in 68 cases of advanced gastric cancer, 40 cases of chronic gastritis, 30 cases of intestinal metaplasia and 20 cases of dysplasia.

RESULTS: MtMSI was observed in 12.5% (5 of 40) of chronic gastritis, 20.0% (6 of 30) of intestinal metaplasia, 25.0% (5 of 20) of dysplasia and 38.2% (26 of 68) of gastric cancer. These findings showed a sequential accumulation of mtMSI in the histological progression from chonic gastritis to gastric cancer. An association of mtMSI with intestinal histological type and distal location was found (P=0.001 and P=0.002), whereas no significant correlation was found between mtMSI and age at diagnosis, sex, tumor size, depth of invasion, lymph node spread and clinical stages (P>0.05).

CONCLUSION: MtMSI may play an early and important role in the gastric carcinogenesis pathway, especially in the intestinal type and distal gastric cancer.

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INTRODUCTION

The mechanisms of carcinogenesis in the gastric mucosa remain unclear. Genetic instability is strongly involved in neoplastic transformation and progression[1-7]. In gastrointestinal carcinomas, such genetic instability may be classified into two different forms in which hypermutability occurs either due to chromosomal instability or due to microsatellite instability (MSI)[8-11]. MSI represents an important form of genomic instability associated with defective DNA mismatch repair in tumors. Although the MSI in nuclear DNA (nMSI) of gastric cancer has been established, little attention was paid to the MSI in mitochondrial DNA (mtMSI) in this cancer. In the present study, we analysed the mtMSI in gastric cancer and its premalignant lesions to elucidate whether mtMSI led to the progression from chronic gastritis to gastric cancer, via intestinal metaplasia and dysplasia.

MATERIALS AND METHODS

Tissue samples

Forty cases of chronic gastritis, 30 cases of intestinal mataplasia and 20 cases of dysplasia obtained from patients undergoing upper endoscopy for dyspepsia and 68 cases of surgically resected gastric cancer tissues were studied. Tissues from non-tumor or non-inflammatory gastric mucosa, showing no dysplasia or metaplasia, were used as a control in analysis of mtMSI. Hematoxylin-eosin (HE) staining was used for the histopathological diagnosis, evaluation and grading of gastritis, atrophy, intestinal metaplasia, dysplasia and cancer. Genomic DNA was isolated by standard proteinase-K digestion and phenol-chloroform extraction protocols. None of the patients with gastric cancer included in the present series had received chemotherapy or radiation therapy before operation.

mtMSI detection

PCR-single strand conformation polymorphism (PCR-SSCP) was performed to amplify the microsatellite sequence of mtDNA using published primers[18]. The primer consisted of 2 D-loop regions and 5 coding regions (Table 1). The reaction conditions and procedures were similar to those reported by Hebano *et al*[12].

Each PCR was digested by appropriate restriction enzymes and electrophoresed at 300 V at 22 ℃ for 2 h on a 75g/L polyacrylamide gel containing 50 mmol/L boric acid, 1 mmol/L EDTA and 25g/L glycerol. After silver staining, PCR products that showed mobility shifts were directly sequenced using an appropriate internal primer and analyzed using the 373A automated DNA sequencer (Perkin Elmer Cetus). All analyses were repeated twice to rule out PCR artifacts.

Table 1 Sequences of primer for PCR analysis

Repeat sequence	mtDNA region	Position	Annealing (℃)	Primer (5'-3')
(C)*n*	270-425	D-loop	58	TCCACACAGACATCAATAACA
				AAAGTGCATACCGCCAAAAG
(CA)*n*	467-556	D-loop	55	CCCATACTACTAATCTCATCAA
				TTTGGTTGGTTCGGGGTATG
(C)6	3 529-3 617	ND1	55	CCGACCTTAGCTCTCACCAT
				AATAGGAGGCCTAGGTTGAG
(A)7	4 555-4 644	ND2	55	CCTGAGTAGGCCTAGAAATAAA
				ACTTGATGGCAGCTTCTGTG
(T)7	9 431-9 526	COIII	55	CCAAAAAGGCCTTCGATACG
				GCTAGGCTGGAGTGGTAAAA
(C)6 and	12 360-12 465	ND5	55	CACCCTAACCCTGACTTCC
(A)8				GGTGGATGCGACAATGGATT
(CCT)3	12 940-13 032	ND5	55	GCCCTTCTAAACGCTAATCC
and (AGC)3				TCAGGGGTGGAGACCTAATT

Statistical analysis

Chi-square test with Yates' correction was used. A P value <0.05 was considered statistically significant.

RESULTS

Sixty-eight gastric cancer samples and 90 benign gastric mucosal lesions were screened for mtMSI at seven repeat sites using the PCR-RFLP method. Figure 1 exhibits a representative mobility-shift band compared with normal counterpart. mtMSI was observed in 26 out of 68 cases (38.2%) of gastric cancer, 5 out of 40 cases (12.5%) of chronic gastritis, 6 out of 30 cases (20%) of intestinal metaplasia, and 5 out of 20 cases (25.0%) of dysplasia (Table 2).

The clinicopathological characteristics of mtMSI-positive cases were compared with those of cases that were mtMSI-negative (Table 3). An association of mtMSI with intestinal histological type and distal location was found (P=0.001 and P=0.002), whereas no significant correlation was found between mtMSI and age at diagnosis, sex, tumor size, depth of invasion, lymph node spread and clinical stages (P>0.05).

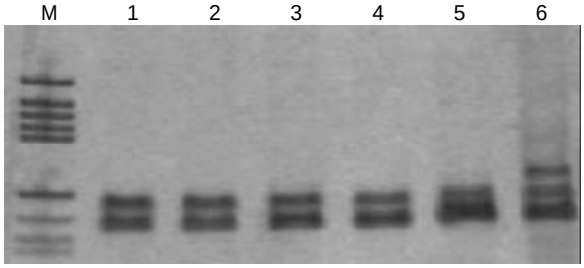

Figure 1 Detection of mitochondrial DNA microsatellite instability in gastric cancer by PCR-single strand conformation polymophism. Lane 6 indicates conformational variants associated with mitochondrial DNA microsatellite instability.

Table 2 mtMSI in gastric cancer and its precursor

	n	mtMSI(%)
Chronic gastritis	40	5(12.5)
Intestinal metaplasia	30	6(20.0)
Dysplasia	20	5(25.0)
Gastric cancer	68	26(38.2)

Table 3 Characteristics of 68 gastric cancer patients

Characteristic	*n*	mtMSI-positive	tMSI-negative
Age			
<40 years	15	5	10
>40 years	53	21	32
Sex			
Male	42	15	27
Female	26	11	15
Size			
<5 cm	38	14	24
>5 cm	30	12	18
Histological type			
Intestinal	41	22[b]	19
Diffuse	27	4	23
Tumor location			
Distal	45	23[c]	22
Proximal	23	3	20
Invasion			
Within the wall	33	12	21
Invading serosa	35	14	21
Lymph node spread			
Absent	30	11	19
Present	38	15	23

[b]P=0.001 *vs* the groups of diffuse type and [c]P=0.002 *vs* the groups of proximal loation.

DISCUSSION

Carcinogenesis is a long-term, multistep process driven by multiple genetic and epigenetic changes in susceptible cells, which gain a selective growth advantage and undergo clonal expansion. Genetic instability is an important factor in the rapid accumulation of these genetic changes. Much attention has been directed to the genetic events in nDNA, such as activation of oncogenes, inactivation of tumor suppressor genes, and defects of mismatched DNA repair genes[13,14]. However, several aspects in the process of carcinogenesis are still unclear. It has been shown that somatic mutations in mtDNA were detected in various human tumors[15-18]. In addition, microsatellite instability has also been shown in mtDNA of colorectal and gastric carcinomas[18-20]. Further studies demonstrated that repeated mononucleotide alteration, missense mutation, and small deletion in NADH dehydrogenase genes and alteration in polycytidine (C)*n* tract in the D-loop region of mtDNA could occur in colorectal carcinomas[18]. These results imply that mtMSI of colorectal carcinomas may likely result from certain deficiencies in DNA repair. Therefore, it has been proposed that somatic mutations and mtMSI play a role in tumorigenesis and development of cancer[21,22]. To study the role of mtMSI in gastric carcinogenesis, we analyzed 68 cases of gastric cancer using seven microsatellite markers known to be altered in gastrointestinal carcinomas. MtMSI was found in 38.2% of patients with gastric cancer, implying that mtMSI may play an important role in the occurrence of a part of gastric cancers.

The majority of gastric carcinomas, particularly the "intestinal" type, which is most common in populations at high risk, were preceded by a precancerous stage, characterized by the following sequencial steps, namely chronic gastritis, intestinal metaplasia, and dysplasia[23,24]. Although numerous cytogenetic and molecular genetic studies have been performed on gastric adenocarcinomas, fundamental data pertaining to precursor lesions which could substantially clarify our understanding of the tumorigenesis in gastric mucosa are not available. This is the first study to examine the frequency of mtMSI in intestinal metaplasia and dysplasia, two premalignant lesions of gastric cancer in individuals without gastric cancer. If mtMSI plays an early and significant role in gastric carcinogenesis, one might expect to find mtMSI in metaplastic and dysplasia tissues before the development of cancer. In this study, mtMSI was detected in 12.5% of chronic gastritis, 20.0% of intestinal metaplasia, and 38.2% of gastric cancer tissues examined. These findings showed a sequential accumulation of mtMSI in the histological progression from chronic gastritis to cancer via intestinal metaplasia and dysplasia, suggesting an early and important role of mtMSI in the gastric carcinogenesis pathway, and they may define a subset of individuals susceptible to gastric cancer.

Cancers from different mutational pathways are thought to have different clinical features. nMSI+ gastric cancer was characterized by older age, antral location, intestinal type, lower prevalence of lymph node metastasis, and a lower pTNM stage[25,26]. However, the clinicopathologic characteristics of mtMSI+ gastric cancers remain unclear. In the current study, we did not find an obvious relationship between mtMSI and tumor size, depth of invasion, node metastasis or clinical stages, indicating a limited role of mtMSI in predicting the prognosis of gastric carcinomas. Gastric carcinomas can be divided into "intestinal" type and "diffuse" type. A distinct genetic pathway has been found in gastric carcinogenesis of different histological subtypes and their tumor progression[27-29]. Increased beta-catenin mRNA levels and mutational alterations of APC and beta-catenin gene were present in intestinal type gastric cancer[30,31], whereas epigenetic inactivation of E-cadherin via promoter hypermethylation might be an early critical event

in the development of undifferentiated tumors[32-35]. In this study, a marked difference in mtMSI was noted in gastric cancer. MtMSI was much more frequent in intestinal-type gastric cancers as compared with diffuse-type gastric cancer, suggesting that mtMSI is a predisposing event in intestinal type of gastric cancer. In contrast to mtMSI-negative gastric cancer, mtMSI-positive gastric tumors tended to exhibit a predominant distal location, similar to nMSI-positive gastric tumors.

The mechanisms underlying mtMSI in gastric mucosa remain unclear. In gastric mucosa, reactive oxygen species (ROS) are commonly released in inflamed gastric mucosa as a result of infection with *Helicobacter pylori* (*H pylori*), especially CagA+ strains, and they might be responsible for mtMSI-positive gastric cancer[36-38]. Mitochondrial genome was particularly susceptible to oxidative damage and mutation because of the high rate of ROS generation and inefficient DNA repair system in the organelle[39,40]. ROS and defective DNA repair were the two causes of increased damage proposed to explain mtMSI in *H pylori* -associated gastric cancer[41-43]. A possibly important role of *H pylori* in the development of mtMSI-positive gastric cancer needed to be further studied.

Although gastric cancer is a common disease, molecular markers for its early diagnosis are lacking. Mitochondrial DNA mutations occurred in a wide variety of cancers and might be usefull in the detection of cancer[44]. Indeed, some authors have implied that mitochondrial genome instability is so common and the enrichment of mutations in cancer is so significant that some mutations probably confer a selective or replicative advantage to those cells that have acquired such mutations[21]. Others have suggested that mtDNA mutations may enhance the toxicity of anti-cancer treatments[45-48]. Thus, the existence of mtDNA mutations in cancer may impact diagnosis and treatment and may be important in understanding the progression of some cancers. Given the early involvement of mtMSI in the multistep gastric carcinogenesis model, detection of mtMSI could serve as a surrogate marker for the risk of gastric cancer development[49]. It might help to identify high-risk patient, by determining mtMSI of preneoplastic lesions, such that close monitoring or potential intervention can be performed. Because the majority of patients with intestinal metaplasia and dysplasia will not progress to cancer and only a proportion of these patients harbore mtMSI, it is conceivable that patients with intestinal metaplasia and dysplasia displaying mtMSI are at greater risk of developing gastric cancer than those without mtMSI.

In conclusion, a high frequency of mtMSI can be found in gastric cancer and its premalignant lesions. Taking into consideration of the progressive increase in mtMSI frequency from premalignant to malignant lesions, our results suggest the early involvement and continuous accumulation of mtMSI in gastric cells that have entered the multistep gastric carcinogenesis pathway. The role of mtMSI in premalignant gastric lesions as a surrogate marker of the risk of gastric cancer development warrants further investigation.

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