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Original Article

The relation of microsatellite instability to expression of hTERT in human gastric carcinoma

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Abstract: Objective: To investigate the role of microsatellite instability (MSI) in the pathogenesis of gastric carcinoma and its relationship with the expression of hTERT gene. Methods: 75 cases of gastric carcinoma and paired normal control tissues were included in this study. MSI of BAT-25, BAT-26, D5S346, D17S250 and D2S1235 were detected by PCR, native polyacrylamide gel electrophoresis, and silver staining while the expression of hTERT was localized by immunohistochemistry at the same time. Results: MSI positive rates of BAT-25, BAT-26, D5S346, D17S250 and D2S123 were 14.7%, 12.00%, 26.67%, 16% and 21.3%. MSI was obviously related with lymph node metastasis and pathologic stages respectively (P<0.05), but not with age, gender, histologic type, or infiltration depth (P>0.05). hTERT was not expressed in normal gastric mucosa, but in intestinal metaplasia, dysplasia, and gastric carcinoma. The positive rate of hTERT was 76% (57/75) in 75 cases of gastric carcinoma tissues. The expression of hTERT was obviously related to histological type (P<0.05), but not to age, gender, lymph node metastasis, depth of invasion, or staging, respectively (P>0.05). The positive rate was higher in poorly differentiated cases than in moderately and well differentiated cases (P<0.05). MSI accounted for 28.1% of 57 hTERT positive cases while MSI accounted for 72.2% in 18 hTERT negative cases. Spearman rank correlation analysis showed that MSI was negatively related to hTERT expression (r=0.387, P=0.001). Conclusion: MSI may play an important role in the pathogenesis and progression of gastric carcinoma by affecting the expression of TERT gene.

Keywords: Gastric carcinoma, microsatellite instability, hTERT

Introduction

The occurrence and development of gastric carcinoma is a complicated and gradual process in which varied genetic changes are involved, including activation of oncogenes and inactivation of tumor suppressor genes. Microsatellite instability (MSI) is genetic instability of length because of insertion or loss of simple repetitive sequences caused by frequent DNA replication errors. Abnormalities of mismatch repair gene may be related to the instability of the tumor genome [1-3]. As the catalytic subunit of telomerase, human telomerase reverse transcriptase (hTERT) determines the activation of telomerase, and so plays an important role in the pathogenesis of neoplasia [4]. Five microsatellite loci including BAT-25, BAT-26, D5S346, D17S250, and D2S123 are recommended by the National Cancer Research Collaborative Group and were selected in this study. By examining the incidence of MSI at these five loci and detecting hTERT expression in gastric carcinoma, we investigated the role of MSI in the pathogenesis of gastric carcinoma and its relationship with hTERT expression.

Materials and methods

Clinical materials

75 cases of gastric carcinoma and the corresponding normal tissues were obtained from surgical resection at three different hospitals in Wuwei City (Gansu, China) from December 2015 to July 2016. All cases were diagnosed to be gastric carcinoma by three experienced pathologists, without preoperative radiotherapy and chemotherapy. Informed consents were signed by all subjects. This study was approved

Table 1. Sequences of PCR primers used at different microsatellite loci

Loci	Primer sequences		Length (bp)
	Sense	Antisense	
Bat-25	5'-TCGCCTCCAAGAATGTAAGT-3'	5'-TCTGCATTTTAACTATGGCTC-3'	~90 bp
Bat-26	5'-TGACTACTTTTGACTTCAGCC-3'	5'-AACCATTCAACATTTTTAACCC-3'	80~100 bp
D5S346	5'-ACTCACTCTAGTGATAAATCG-3'	5'-AGCAGATAAGACAGTATTACTAGTT-3'	96~122 bp
D17S250	5'-GGAAGAATCAAATAGACAA-3'	5'-GCTGGCCATATATATATTTAAACC-3'	~150 bp
D2S123	5'-AAACAGGATGCCTGCCTTTA-3'	5'-GGACTTTCCACCTATGGGAC-3'	197~227 bp

by the Ethics Committee of San Ai Tang Hospital and performed in accordance with the ethical guidelines of the Declaration of Helsinki. Some of the tumor tissues and corresponding normal tissues of margin (more than 5 cm distant from the tumor) were stored immediately at -70°C for DNA extraction, and others were fixed in 10% neutral buffered formalin and embedded in paraffin for H&E and immunohistochemical staining after the removal of hemorrhagic and necrotic tissues. Fifteen cases of normal gastric mucosa were used as controls in immunohistochemistry.

According to TNM staging criteria of 2010 International Union Against Cancer (UICC)/ American Joint Committee on Cancer (AJCC), 35 cases were stage I/II and 40 cases were stage III/IV. According to 2010 WHO histologic classification criteria, 7 cases were well-differentiated with 20 cases moderately-differentiated and 30 cases poorly-differentiated, and 18 cases were mucinous adenocarcinoma. 57 cases were males and 18 cases were females. All patients were from 31 to 76 years old with a median age of 55.7.

MSI testing

DNA extraction was performed as described in the instructions of the DNA extraction kit (TaKaRa, Japan). The primers of five microsatellite loci including BAT-25, BAT-26, D5S346, D17S250 and D2S123 were synthesized as described in the literature [5] by Shanghai Sangon Biotech Company (**Table 1**). The microsatellite DNA was amplified by the PCR thermal cycler (Santa Cruz, USA) for 30 cycles. Circulation parameters were as follows: 95°C for 30 s, 56°C for 30 s, 72°C for 30 s. Reaction mixture included 2.5 μl of 10× Buffer (TaKaRa, Japan), 2.0 μl of dNTP (TaKaRa, Japan), 1 μl of sense primer, 1 μl of antisense primer, 1 μl of genomic DNA, 0.125 μl of Tap DNA polymerase (TaKaRa, Japan), and then deionized water was added to 25 μl of total volume. PCR products were dissolved in the single chain loading buffer including 980 ml/L deionized formamide (Shanghai Chemical Reagents Co. Ltd), 20 mmol/L EDTA (Shanghai Chemical Reagents Co. Ltd), 0.1 g/L bromophenol blue, 0.1 g/L xylene green (Shanghai Chemical Reagents Co. Ltd). After incubation at 97°C for 7.5 min, samples underwent non-denaturing polyacrylamide bilayer gel (28.5:1.5) electrophoresis containing 5% glycerin at 4°C and 180 V for 3 h.

Immunohistochemical staining

Tissue blocks were cut into 4 μm thickness, deparaffinized in xylene, rehydrated with graded alcohols. Hematoxylin & eosin (H&E) staining was performed as formal. Heat-induced epitope retrieval was performed using a steamer. Immunostaining was performed with rabbit anti-hTERT monoclonal antibody (1:100, Santa Cruz, USA). Sections were stained with a streptavidin-peroxidase (SP) kit (Maixin, China), and diaminobenzidine tetrahydro-chloride substrate (Maixin, China) was used as the chromogen. Sections were then slightly counterstained with hematoxylin, dehydrated, cleared, and mounted. Appropriate positive and negative controls were included.

Result determination criteria

Compared with normal controls, cases with band insertion or shift were judged to be positive while cases with the same band distribution were judged to be negative in the electrophorogram [6]. All images subjected to H&E staining and IHC were viewed under light microscopes (Nikon ECLIPSE 80i, Japan). The results were evaluated by three experienced pathologists. Cases with brownish granules at the membrane and/or in the cytoplasm were judged to be positive.

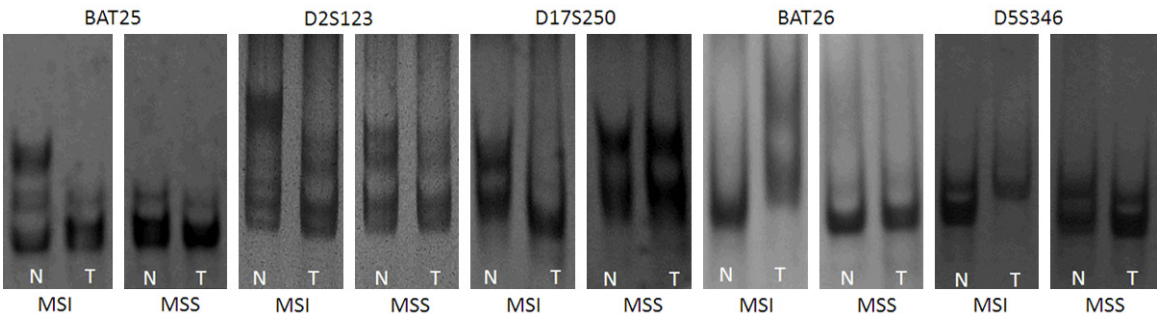

Figure 1. Polyacrylamide gel electrophoresis of PCR products at 5 microsatellite loci in gastric carcinoma (MSI, microsatellite instability; MSS, microsatellite stability; N, paraneoplastic normal tissue; T, gastric carcinoma). Symmetrically distributed bands are shown in MSS-N. Loss or insertion of bands is shown in MSI-T.

Table 2. The relation between MSI and different clinicopathologic features in gastric carcinoma

Clinicopathologic feature	n	MSS	MSI	χ^2	P
Average age	75	56.51±10.59	55.87±10.52	1.043	0.594
Gender					
Male	57	35	22	3.331	0.191
Female	18	11	7		
Histologic type					
Well differentiated	7	3	4	16.803	0.157
Moderately differentiated	20	12	8		
Poorly differentiated	30	17	13		
Mucinous adenocarcinoma	18	14	4		
Lymph node metastasis					
No	17	5	12	10.457	0.005
Yes	58	41	17		
Infiltration depth					
With serosa breakthrough	47	26	21	2.065	0.356
Without serosa breakthrough	28	20	8		
TNM stages					
I+II	35	11	24	14.776	0.001
III+IV	40	35	5		

Statistical analysis

SPSS13.0 statistical software (SPSS Inc., Chicago, IL, USA) was used to analyze the data. The test level was α=0.05, and P<0.05 was statistically significant.

Results

The positive rate of MSI and its relation with clinicopathological features in gastric carcinoma

The positive rates of MSI at microsatellite loci as BAT-25, BAT-26, D5S346, D17S250 and D2S123 were 14.7% (11/75), 12.00% (9/75), 26.67% (20/75), 16% (12/75) and 21.3% (16/75) in 75 cases of gastric carcinoma, respectively (**Figure 1**). MSI was related to lymph node metastasis and pathological stages respectively (P<0.05), but not to age, gender, histologic type, and infiltration depth (P>0.05) (**Table 2**).

Expression of hTERT protein and its relation to clinicopathological features in gastric carcinoma

hTERT was not expressed in normal gastric mucosa, but in intestinal metaplasia, dysplasia, and gastric carcinoma with about 76%, 60%, and 25% positive rates (**Table 3**). Brown

Table 3. Positive rates of hTERT protein in different tissues

Type	n	Positive (%)
Normal gastric/intestinal	25	0 (0)
Intestinal metaplasia	20	5 (25)
Atypical hyperplasia	20	12 (60)
Gastric cancer	75	57 (76)

particles were mainly distributed in the cytoplasm, occasionally in the nucleus (**Figure 2**). The expression of hTERT was related to histologic type (P<0.05), but not with age, gender, lymph node metastasis, depth of invasion and staging, respectively (P>0.05). The positive rate was higher in poorly differentiated cases than in moderately and well differentiated cases (P<0.05) (**Table 4**).

The relation of MSI with hTERT expression in gastric carcinoma

MSI accounted for 28.1% (16/57) while MSS accounted for 71.9% (41/57) of 57 hTERT positive cases while MSI accounted for 72.2% (13/18) while MSS accounted for 27.8% (5/18) in 18 hTERT negative cases. Spearman rank correlation analysis showed that MSI was negatively related to hTERT expression (r=-0.387, P=0.001) (**Table 5**).

Discussion

Microsatellite instability (MSI) is a simple repeat sequence caused by false replication, which may be related to DNA mismatch repair gene defects. Mismatch repair has the function of maintaining genomic stability and reducing spontaneous mutation. Defects lead to genomic instability and susceptibility to tumors [7, 8]. Recent studies have found that MSI is one of the molecular mechanisms leading to tumorigenesis and progression, and is also an important marker of tumor cells [9, 10]. MSI is preserved in the genome by DNA replication and cell division. It can increase the instability of other genes, result in instability of the entire genome, increase spontaneous mutation of cells, lead to cell proliferation and dysplasia, and therefore promote tumorigenesis [11, 12].

Studies have confirmed that gastric carcinoma has higher incidence of MSI than any other tumor. MSI may be the early molecular events in the multi-step progression of gastric carcinoma [13]. The incidence of MSI was 13.0-44.0% in sporadic gastric carcinoma [14]. MSI is closely related to the clinicopathologic behavior of gastric carcinoma. Incidence of MSI-H is high in 1/3 gastric carcinoma and intestinal metaplasia, and MSI is more common in the early stages of TNM staging [15, 16]. There were no significant differences in tumor location, age, or gender in 128 cases of sporadic gastric carcinoma between cases with MSI-H and MSS/MSI-L [17].

We found that the positive rates of MSI at microsatellite loci as BAT-25, BAT-26, D5S346, D17S250, and D2S123 were 14.7%, 12.00%, 26.67%, 16% and 21.3% in 75 cases of gastric carcinoma, respectively. The positive rate of MSI was higher in cases with lymph node metastasis than those without lymph node metastasis, and was higher than in cases at stage I/II than those at stage III/IV. These results were almost the same as the results of some researchers [18, 19]. The difference may be related to the genetic background, the numbers of subjects, the location and numbers of selected microsatellite loci, and the selected population.

As a regulatory subunit of telomerase, hTERT not only relies on and has some limiting effect on the activity of telomerase, but also plays an important role in the development of tumors [20]. The expression of hTERT gene determines the activation of telomerase. With its own RNA as template, telomerase can consecutively synthesize new telomeric DNA sequences (TTAGGG repeats) which are added to the end of the chromosome to compensate for telomere loss and to prevent telomere from shortening, so gene stability is kept. Telomere dysfunction is associated with a variety of tumors [21]. Expression of hTERT in gastric carcinoma and precancerous lesions increased with the activity of telomerase and was related to differentiation of tumor. A high level of expression existed in poorly differentiated tumors, suggesting that the increased expression of hTERT and telomerase activity may be associated with the development of gastric carcinoma [22, 23].

In this study, hTERT was not expressed in normal gastric mucosa while there was a gradual increasing trend in intestinal metaplasia, dysplasia and gastric carcinoma. The expression of hTERT was obviously related to histologic

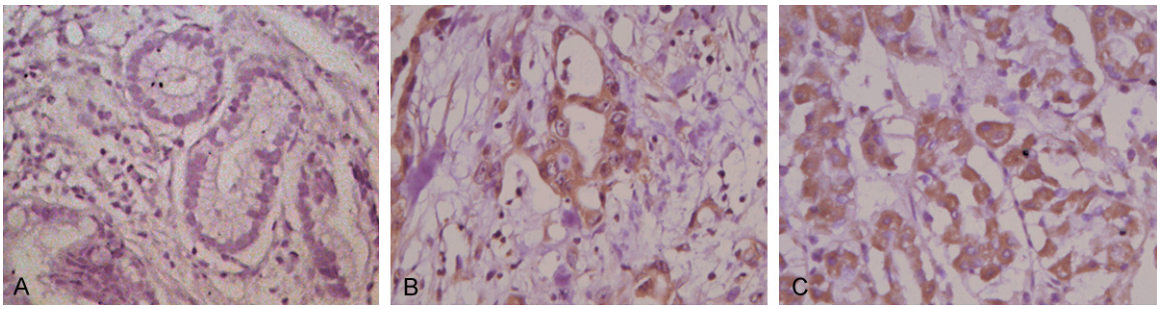

Figure 2. Immunohistochemical localization of hTERT in gastric carcinoma. A. hTERT was not expressed in gastric mucosa (SP, ×400). B. hTERT was expressed in the cytoplasm and perinuclear area of tumor cells in gastric adenocarcinoma with MSI (SP, ×400). C. hTERT was expressed in cytoplasm and perinuclear area of tumor cells in gastric adenocarcinoma with MSS (SP, ×400).

Table 4. The relation between hTERT protein and different clinicopathologic features in gastric carcinoma

Clinicopathologic feature	n	Positive	χ^2	P
Average age				
<55	32	21	1.651	0.199
≥55	43	35		
Gender				
Male	57	45	0.558	0.455
Female	18	12		
Histologic type				
Well differentiated	7	3	13.914	0.003
Moderately differentiated	20	11		
Poorly differentiated	30	27		
Mucinous adenocarcinoma	18	6		
Lymph node metastasis				
No	17	10	2.442	0.118
Yes	58	47		
Infiltration depth				
With serosa breakthrough	47	33	1.540	0.215
Without serosa breakthrough	28	24		
TNM stages				
I+II	35	27	1.295	0.255
III+IV	40	33		

Table 5. The relation of MSI with expression of hTERT in gastric carcinoma

hTERT	n	MSI (29)	MSS (46)
Positive	57	28.1 (16/57)	71.9 (41/57)
Negative	18	72.2 (13/18)	27.8 (5/18)

r=0.387, P=0.001.

type, but not to age, gender, lymph node metastasis, depth of invasion and staging, respectively. The positive rate was higher in poorly differentiated cases than in moderately and well differentiated cases. The results suggested that hTERT may play an important role in the growth and infiltration of tumor and in the development of gastric carcinoma.

Yasuhiro et al. found that the positive rate of MSI was 48% while the positive rate of MMS was 86% in patients with gastric carcinoma who were positive for hTERT detection [24]. In this study, however, MSI accounted for 28.1% with MSS accounting for 71.9% in 57 hTERT positive cases; while MSI accounted for 72.2% with MSS accounting for 27.8% in 18 hTERT negative cases. Spearman rank correlation analysis showed that MSI was negatively related with hTERT expression. The results suggest that MSI may affect the expression of hTERT.

Conclusion

In summary, MSI may play an important role in the occurrence and progression of gastric carcinoma and MSI may affect the expression of hTERT.

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Disclosure of conflict of interest

None.

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