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RAPID COMMUNICATION

Role of detection of microsatellite instability in Chinese with hereditary nonpolyposis colorectal cancer or ordinary hereditary colorectal cancer

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Abstract

AIM: To detect microsatellite instability (MSI) in patients with hereditary nonpolyposis colorectal cancer or ordinary hereditary colorectal cancer and to provide criteria for screening the kindreds with hereditary nonpolyposis colorectal cancer at molecular level.

METHODS: MSI was detected in the specimens from 20 cases with HNPCC, 20 cases with ordinary hereditary colorectal cancer and 20 cases with sporadic colorectal cancer by means of polymerase chain reaction-single strand conformation polymorphism.

RESULTS: The positive rate of MSI was 85% (17/20) in HNPCC group, 40% (8/20) in ordinary hereditary colorectal cancer group and 10% (2/20) in the sporadic colorectal cancer group respectively. The differences were significant. The mean ages of the three groups were 43.6, 52.2, and 61.8 years respectively, which increased gradually. The incidence of right hemicolon cancer was 64.7%, 37.5%, and 0% respectively, which decreased gradually and had significant difference. The expression ratio of BAT26 and BAT25 was 94.1% respectively, which was highest in the 5 gene sites studied. The incidence of poorly differentiated adenocarcinoma was 70.6% in HNPCC group among high frequency microsatellite instability (MSI-H), which was higher than the other two groups, which had 50% and 50% respectively.

CONCLUSION: The incidence of MSI-H is higher in HNPCC group. The detection of MSI is simple and economical and has high correlation with the clinicopathologic feature of HNPCC and can be used as a screening method to detect the germ line mutation of the mismatch repair gene.

Key words: Hereditary nonpolyposis colorectal cancer; Microsatellite instability; Ordinary hereditary colorectal cancer; Single strand conformation polymorphism; Polymerase chain reaction

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INTRODUCTION

Hereditary nonpolyposis colorectal cancer (HNPCC) is an autosomal dominant inheritance syndrome, with a penetrance as high as 70%-80%[1], and accounts for about 5%-15% of all colorectal cancer[2]. The molecular genetic basis of the disease is germ line mutation of the mismatch repair (MMR) gene, which causes failure of the DNA MMR system to repair errors that occur during the replication of DNA and results in alterations in the length of simple, repetitive microsatellite sequences and so called microsatellite instability (MSI). MSI may reflect the mutation of the MMR gene indirectly and can be used as a means of screening gene mutation of the MMR gene[3,4]. Recently, studies showed most patients with HNPCC have MSI[5,6] and the ratio is higher than that of patients with sporadic colorectal cancer[7,8]. In the current study, we tested microsatellites of the former paraffin-embedded tissue by the method of polymerase chain reaction-single strand conformation polymorphism(PCR-SSCP) of the Chinese patients who fulfilled the criteria for HNPCC and ordinary hereditary colorectal cancer and tested its application value in the clinic.

MATERIALS AND METHODS

Patients

HNPCC group (group A): 20 patients (12 men, 8 women, mean age 48 years, range 32-70 years) who fulfilled the criteria for HNPCC of Chinese people[9] were selected and their family histories were obtained by follow-

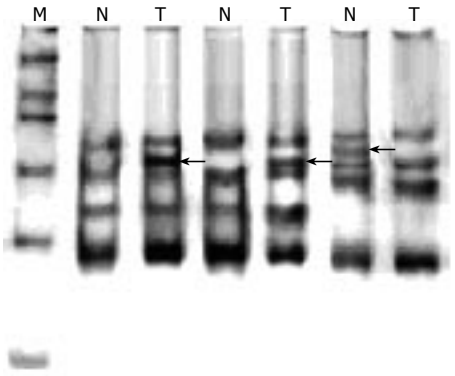

Figure 1 Positive results of MSI. (arrowhead indicates positive results). M: normal DNA; N: normal tissue; T: cancer tissue.

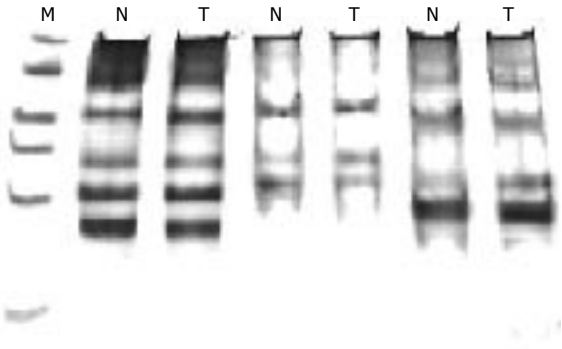

Figure 2 Negative results of MSI (results of 3 pairs are all negative). M: normal DNA; N: normal tissue; T: cancer tissue.

up study. Among them 9 cases were with carcinoma of ascending colon, 2 cases with carcinoma of transverse colon, 1 case with carcinoma of descending colon, 2 cases with carcinoma of sigmoid colon and 6 cases with carcinoma of rectum.

Ordinary hereditary colorectal cancer group (group B): 20 patients (13 men, 7 women, mean age 61 years, range 30-83 years) who fulfilled the criteria for ordinary hereditary colorectal cancer of Chinese people[10] were selected. Among them 5 cases were with carcinoma of ascending colon, 3 cases with carcinoma of transverse colon, 1 case with carcinoma of descending colon, 2 cases with carcinoma of sigmoid colon and 9 cases with carcinoma of rectum.

Sporadic colorectal cancer group (group C): 20 patients (10 men, 10 women, mean age 65 years, range 42-80 years) who were diagnosed with colorectal cancer by pathology and with no family history were selected. Among them 5 cases were with carcinoma of ascending colon, 4 cases with carcinoma of sigmoid colon and 11 cases with carcinoma of rectum.

Methods

For MSI analysis, normal and tumor tissues of the three groups were embedded with paraffin, 4-5 slides of tissue with thickness of 4 μm were sliced and stained with HE. Normal and tumor tissues were selected with microscopy. They were transferred to the EP tubes which contained 150 μL cell lysates. Then DNAs of the normal and tumor tissues were extracted with DNA extraction kit. The primers of the 5 microsatellite loci of HNPCC (BAT26, BAT25, D2S123, D5S346 and D17S250) were selected according to recommendation of the international cooperation organization (Table 1). The primers were synthesized by TAKARA Company.

The reaction system: It was consisted of a total volume of 25 μL with 3 μL template DNA, 0.5 μL forward and reverse primers, 10 × Buffer 2.5 μL, dNTP 2.0 μL, TaqE 0.2 μL, DMSO 1.0 μL, ddH_2O 15.3 μL. The PCR program started with a 95℃ denaturation for 5 min, then at 94℃ for 40 s, 53℃ for 30 s followed by 35 cycles of annealing at 72℃ for 1 min, and extension at 72℃ for 40 s, finally an extension at 72℃ for 10 min. The PCR products were analyzed by electrophoresis on 1.5% agarose gels containing ethidium bromide.

Detection of MSI: Single strand conformation polymorphism (SSCP) was used to analyze MSI. The PCR products of normal and tumor tissues were mixed with the same volume of alkaline buffer, then started with 97℃ denaturation for 10 min and put in the mixture of water and ice for 5 min. Then electrophoresis was performed vertically on the 10% nondenaturing polyacrylamide gel (constant power; 60 W) for about 4 h. When the indicating straps reached the inferior margin of the gel or the tracer agent disappeared, silver staining, coloration, fixation and termination were performed. When the film dried 24 h later, the imaging was observed.

Results assessment: MSI was defined by the presence of novel bands following PCR amplication of tumor DNA, which were not present in PCR products of the corresponding normal DNA. If more than 2 of them showing positive results, it was defined as high frequency microsatellite instability (MSI-H), and if only 1 of them showing positive result, it was defined as low frequency microsatellite instability (MSI-L), and if none of them showing positive result, it was named microsatellite stable (MSS).

RESULTS

The results of detection of MSI are shown in Figures 1 and 2. and Tables 1 and 2.

The relationship among MSI-H, mean age of the patients and the sites of cancer are shown in Table 3.

The ratio of every locus occupying in the MSI-H is shown in Table 4.

The clinical pathological characteristics of colorectal cancer are as follows: in the 17 cases with MSI-H of group A, 7 cases had multiple cancers, among which 4 cases were with multiple cancers simultaneously, 3 cases were with multiple cancers at different times. As to groups B and C, none of the patients were with multiple cancers. There were 70.6% (12/17) of poorly differentiated adenocarcinoma in group A, which was more than that of groups B (50%, 4/8) and C (50%, 1/2).

DISCUSSION

Microsatellites are long stretches of apparently redundant DNA between genes by 2-6 nucleotides tandemly arrayed, within which repetitive sequences may be found. Repetition of two bases(CA/GT) is most common. Under normal conditions, the repetitive sequence is constant but

Table 1 Primer sequence of MSI and annealing temperature

Locus	Primer sequence	Annealing temperature (℃)
BAT26	TGACTACTTTTGACTTCAGCC AACCATTCAACATTTTTAACCC	53.9
BAT25	TCGCCTCCAAGAATGTAAGT TCTGCATTTTAACTATGGCTC	53.4
D2S123	AAACAGGATGCCTGCCTTTA GGACTTTCCACCTATGGGAC	57.4
D5S346	ACTCACTCTAGTGATAAATCGGG AGCAGATAAGACAGTATTACTAGTT	54.0
D17S250	GGAAGAATCAAATAGACAAT GCTGGCCATATATATATTTAAACC	53.6

Table 2 Detection of MSI in three groups (%)

Group	*n*	MSI-H	MSI-L	MSS
A	20	85 (17/20)	10 (2/20)	5 (1/20)
B	20	40 (8/20)	35 (7/20)	25 (5/20)
C	20	10 (2/20)	30 (6/20)	60 (12/20)

MSI-H: high frequency microsatellite instability; MSI-L: low frequency microsatellite instability; MSS: microsatellite stable.

highly polymorphic and is an ideal mark for localization of genes. Cells lacking normal DNA mismatch repair acquire mutations that change the length of nucleotide repeat sequences, termed microsatellite instability (MSI). MSI is the pattern of manifestation of MMR and has a close relationship with HNPCC[11,12], therefore the mutation of MMR results in the incidence of MSI. About 70%-90% of the patients with HNPCC had MSI and only 10%-15% occurred in patients with the sporadic colorectal cancer. Although the Amsterdam Criteria (the International Collaborative Group established in 1991) is now used as the diagnostic criteria for HNPCC clinically, its determination depends mainly on family history and clinical symptoms and cannot reflect the true morbidity of the disease and predict the occurrence of HNPCC. Therefore the detection of mutation of MMR genes has become the golden standard for diagnosis of HNPCC. However, the MMR gene is very large, it has no mutational hot spot, and the cost of detection is expensive, thus it cannot be used as a routine means for screening of HNPCC family at present. As a phenotype of MMR gene mutation, MSI can be used as criterium for detection of HNPCC, especially in patients whose family history is not complete and who do not fit the criteria of HNPCC clinically. Therefore, DNA is extracted from the tumor tissue for detection of MSI. When a patient of MSI-H is diagnosed, detection of MMR gene should be done. As to the choice of loci of the microsatellite instability, 5 loci of microsatellite are used for PCR amplification according to the recommendation of the International Collaborative Group and the International Cancer Research Institute and the Collaborative Group of the Chinese Genetic Colorectal Cancer. However, number of the microsatellites detected is not constant, from less than 3 loci to more than 100 loci. The criteria for the number and ratio of the positive locus of MSI tumor are not uniform. Therefore the results of many different researchers could not be compared with each other.

Table 3 Relationship among MSI-H, mean age of the patients and the sites of cancer (%)

Group	*n*	MSI-H	Mean age (yr)	Right hemicolon	Left hemicolon
A	20	17 (85)	43.6	11 (64.7)	6 (35.3)
B	20	8 (40)	52.2	5 (62.5)	3 (37.5)
C	20	2 (10)	61.8	0 (0)	2 (100)

Table 4 Ratio of every locus occupying in the MSI-H (%)

Locus	Positive ratio of MSI-H	Positive ratio of MSI
BAT26	94.1 (16/17)	85 (17/20)
BAT25	94.1 (16/17)	90 (18/20)
D2S123	64.7 (11/17)	55 (11/20)
D5S346	52.9 (9/17)	50 (10/20)
D17S250	35.3 (6/17)	40 (8/20)

In this study, we amplified 5 loci of microsatellite by PCR according to the criteria of the International Collaborative Group and the International Cancer Research Institute and found the positive ratio was as high as 85% for MSI-H patients with HNPCC, which is in accordance with other research. We found the 5 loci were ideal for detection of MSI of HNPCC patients. It reflected the mutation of MMR gene objectively and could be used as a reliable indicator for screening of HNPCC family just before gene sequencing. If other loci were selected, the type of MSI could be decided by the percentage of positive locus, and MSI-H could be diagnosed when the percentage of positive loci was more than 30%-40% while MSI-L was diagnosed when the percentage of positive loci was less than 30%. As to which loci should be selected, there are a variety of viewpoints. Some scholars think 1-2 microsatellite loci are enough. Hoang[13] believes BAT26 has high correlation with MSI, and their concordance rate is very high. Because the detecting process is relatively simple, Stone[14] detected MSI from tumor specimen using BAT26 directly and recommended that it should be used more extensively. Our study found the positive rate of BAT26 was as high as 94.1%, indicating its high sensitivity for MSI-H detection. At the same time, the positive rate of BAT25 was also 94.1%. Their annealing temperature was close to each other and could be carried out in the same reaction system. The combined application of BAT26 and BAT25 may elevate the specificity of the experiment.

Moreover, our research found that about 40% of the patients with ordinary hereditary colorectal cancer were classified as MSI-H, although it was lower than that of the HNPCC group, it was higher than that of the sporadic colorectal cancer group (the positive rate was 10%), suggesting that about 30% of the patients with ordinary hereditary colorectal cancer had the characteristics of HNPCC. Whether these patients were potential HNPCC

patients needs further study. MSI has been shown to have a close relationship with the onset age of the patients in many recent studies. MSI-H shows good consistency with early onset of age. Edmonston[15] found that the patients whose onset of age was less than 41 years were all MSI-H positive, and the youngest onset age of MSI-H was less than 26 years. Terdiman[16] found that in the different HNPCC families, the final diagnosis age of MSI-H was young and there were more family members with HNPCC than other families, and the ratio of multiple cancers or other HNPCC-related cancer increased. Our research revealed that the mean onset age of MSI-H in the HNPCC group was 43.6 years, which was younger than that of the ordinary hereditary colorectal cancer group (52.2 years) and the sporadic colorectal cancer group (61.8 years). The incidence of multiple cancers or other HNPCC-related cancers of HNPCC group was higher than other groups. In addition, MSI had a close relationship with the sites of carcinoma. The carcinoma of MSI-H, either of HNPCC or of sporadic colorectal cancer, was all likely to occur at the proximal half of a colon. Just as Altonen's study showed[17], 62% of the tumors occurred at the proximal hemicolon of the patients with HNPCC while 38% occurred at the distal hemicolon, suggesting that MSI had a close relationship with cancer of right hemicolon and may play a fundamental role in the pathology of cancer of right hemicolon. Our research found the incidences of right hemicolon cancer of the HNPCC group and the ordinary hereditary colorectal cancer group were 64.7% and 62.5% respectively, which were higher than those of left hemicolon cancer. There was no cancer of right hemicolon found in the sporadic colorectal cancer group, because the occurrence of rectum cancer was higher in North China and the bias was related with the cases selected. Our research suggests that MSI had high correlation with the clinical and pathological characteristics of the HNPCC.

At present, there have been few reports about MSI in typical cases of Chinese people and no clinical data about MSI in patients with ordinary hereditary colorectal cancer. Our research demonstrates that the incidence rate of MSI-H in patients with HNPCC, the mean onset age, the site of carcinoma occurrence, and the clinical and pathological characteristics of patients with HNPCC are all different from that of patients with sporadic colorectal cancer. Ordinary hereditary colorectal cancer is a special type between HNPCC and sporadic colorectal cancer. It has partial characteristics of HNPCC but is not the same as HNPCC and has many differences from sporadic colorectal cancer. Whether it has a special molecular genetic mechanism needs further study. In addition, the survival rate of patients with HNPCC is higher than that of patients with sporadic colorectal cancer, which is due to the early diagnosis of some patients with HNPCC[18,19]. Recently, studies reported that MSI induced by abnormal expression of MMR may affect the therapeutic effect of chemotherapy and the prognosis of patients with colorectal cancer[20-22]. Therefore, as an important screening method for HNPCC, MSI has a promising prospect in clinical application.

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