CLINICOPATHOLOGICAL FEATURES OF RER$^+$ COLORECTAL CARCINOMA

XU Ning 徐宁 [1], DING Yan-Qing 丁彦青 [1], XU Li 徐莉 [1], Qiu Hong-ming 邱红明 [2]

[1] *Department of Pathology, Nanfang Hospital, First Military Medical University, Guangzhou 510515*
[2] *Cancer center of Texas university, USA*

ABSTRACT

Objective: Replication errors (RER) is related to initiation and development of colorectal carcinoma (CRC). To investigate the different biological behavior of RER$^+$ and RER$^-$ CRC. Methods: Silver staining PCR-single strand conformation polymorphism (PCR-SSCP) and denatured polyacrylamide gel electrophoresis methods were used to detect microsatellite instability (MSI) at 4 loci on chromosome 2, 5, 17 in paraffin-embedded specimens of 60 colorectal carcinoma (CRC) and their paired normal tissue. RER$^+$ was scored if 2 or more loci behaved as gaining extra bands. Results: The results showed that RER$^+$ was found in 19/60 CRC, among which 7 cases had a family history. According to the criteria of Amsterdam, 4 were diagnosed as hereditary nonpolyposis colorectal cancer (HNPCC), and of which 3 cases were RER$^+$. The ratio RER$^+$ in HNPCC (75%) was significantly higher than that among sporadic CRC (28.5%). Most of the RER$^+$ CRC have the feature of poorly differentiated adenocarcinoma (P<0.01), the tendency to involve the right side of the colon (P<0.05), a higher proportion with a family history (P<0.05), Duckes' A and B stage (P<0.05). Conclusion: The results indicated that RER$^+$ is a relatively common molecular event in CRC. There are different clinico-pathological features and behavior between RER$^+$ and RER$^-$ CPC.

Key words: HNPCC, Colorectal carcinoma, Clinico-pathology, Replication errors.

Microsatellites (MS) are simple repetitive sequences of DNA, they are scattered throughout the genome. Most of them are binucleotide or trinucleotide repeats. Microsatellite instability (MSI) implies that the microsatellite DNA varies in length between tumor and its paired normal tissue. Loeb has indicated that if a true mutator phenotype exists in a subset of tumor cells, the responsible defect is likely to cause MSI. Tumors with MSI at 2 or more loci of all detected loci have been thought to manifest replication errors (RER).[1,2] In this study, we applied PCR-single strand conformation polymorphism (PCR-SSCP) method to detect MSI in 60 colorectal carcinomas (CRC). The relationship between RER$^+$ and CRC tumorigenesis was discussed.

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Correspondence to: DING Yan-qing; Department of Pathology, Nanfang Hospital, First Military Medical University, Guangzhou 510515, China; E-mail: dyq@ timmu.edu.cn

MATERIALS AND METHODS

Specimens

Specimens were obtained from 60 patients with colorectal carcinoma (CRC). Among the patients <50 year old: 30 cases; >50 year old: 30 cases; left CRC: 30 cases; right CRC: 30 cases. It was decided to detect MSI at 4 loci on chromosome 2, 5, 17 in paraffin-embedded specimens. All cases were from Department of Pathology, Nanfang Hospital between 1995–1997 and grouped according to age, sex, family history, Dukes' stage, location and differentiation. All colorectal carcinomas and their paired normal tissue were identified by pathologists.

Diagnostic Criterion of Hereditary Nonpolyposis Colorectal Cancer (HNPCC)

According to Amsterdam criteria.[3]

DNA Isolation

According to literature.[4]

PCR Amplification

PCR was used to amplify MS DNA at 4 loci on chromosome 2, 5, 17 in paraffin-embedded specimens of 60 CRCs and their paired normal tissues.[5] PCR products were loaded on 1% agarose gel and electrophoresized. Only those cases with amplified specific band were used for further MSI analysis.

PCR-SSCP[5] and PCR-denatured PAGE Methods to Detect MSI

Denatured PAGE: PCR products were electrophoresised in 8% polyacrylamide gels containing 8 mol/L urea. The remaining were the same as PCR-SSCP method.

MSI

When the bands between tumor and its corresponding normal tissue DNA were the same size at these MS loci, it was considered as MSI negative (MSI^-). If the size of these loci was changed, either becoming larger or smaller, compared with it's corresponding normal DNAs, MSI was defined (Figure 1, 2). Those MS^+ cases were proved by PCR amplification again.

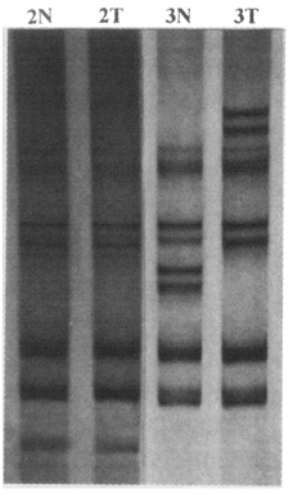

Fig. 1. Silver staining PCR-SSCP
Abnormal bands were shown in sample 3 and 3 sample showed MSI^+. 2 sample showed MSI^-. N tumor margin resection; T tumor tissues.

Statistical Analysis

X^2 test was used. (software: STAT V3.0)

RESULTS

Positive Rate by Using PCR-SSCP and PCR-denatured PAGE

RER^+ was demonstrated in 21.9% (13/60), 16.7% (10/60) respectively, by using PCR-SSCP method or PCR-denatured PAGE. RER^+ was 31.7% (19/60) by using the two methods. The according ratio of MSI of 4 loci detected by both methods was 84.5%. The positive rate of RER^+ could be improved by using the two methods.

The RER^+ was observed in 19 /60 (31.7%) CRC. The ratio of RER^+ patients in HNPCC (3/4, 75%) was significantly higher than that among sporadic CRC (16/56, 28.5%). It was predominantly a feature of poorly differentiated adenocarcinoma ($P<0.01$), a tendency to involve the right side of the colon ($P<0.05$), a higher proportion of family history ($P<0.05$), and Dukes' A, B stage ($P<0.05$) (Table 1).

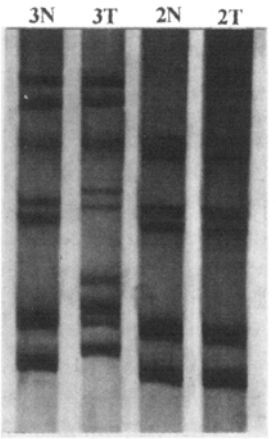

Fig. 2. Silver staining PCR-denatured PAGE
Abnormal bands were shown in sample 3 and 3 sample showed MSI^+. 2 sample showed MSI^-. N tumor margin; T tumor tissues.

Table 1. Comparison of RER^+ CRC in clinical pertinence

Index of clinical pertinence	n	RER^+ N	RER^+ %	*P*
Family history				
+	7	5	71.4%	<0.05
−	53	14	26.4%	
Differentiation				
Poor*	17	11	64.7%	<0.01
Differentiated**	43	8	18.2%	
Location				
Right	30	14	46.7%	<0.05
Left	30	5	16.7%	
Dukes' stage				
A, B	36	15	41.7%	<0.05
C, D	24	4	16.7%	

*Poorly differentiation: It indicates poorly differentiated adenocarcinoma, undifferentiated adenocarcinoma, muci-nous adenocarcinoma, and signet ring adenocarcinoma.
**Differentiation: It indicates mammilla adenocarcinoma, moderately differentiated adenocarcinoma, well differen-tiated adenocarcinoma, and cancer from adenoma.

DISCUSSION

Generally, MSI is detected by ^{32}p labelling in PCR process, followed by electrophoresis in sequencing gel and autoradiography. This technique is highly sensitive and reliable, but it needs specific instru-ments (sequencing gel unit) as well as an isotope which is harmful to human health. SSCP was developed by Orital

et al. (1989) as a means of detecting mutated sequence. This method is based on the fact that the electrophoretic mobility of a single-stranded nucleic acid in a nondenatured polya-crylamide gel depends not only on its size but also on its secondary structure decided by nucleic acid sequence. The defect is that only 60—80% point mutation was detected by PCR-SSCP.[6] When MSI occurred, the length of MS marker will be change, certainly their secondary structure will changed subsequently. According to these reason we applied PCR-SSCP technique to detect MSI, to try to find a simple, sensitive and reliable method which is competitive to the sequencing gel electrophoresis. By using PCR-SSCP combined with silver staining, 13/60 cases of CRC were found to have one or more MS loci with instability.

We also applied PCR-denatured PAGE by using 20 cm in length gel followed by silver staining to detect MSI. The effect is infected by the length of gel and the time of the electrophoresis. The difference of single-stranded nucleic acid length between tumor and corresponding normal tissue of MSI^+ case can be clearly demonstrated, suggesting the alterations of the simple repetitive sequences of the $(CA)_n$ or $(AT)_n$. The abnormal shifting of the single-stranded DNA was showed clearly by both SSCP and denatured polyacrylamide gel electrophoresis methods. The according ratio of MSI of 4 loci detected by both methods was 84.5%, suggesting both methods are sensitive, specific and reliable. Using both methods could improve positive ratio of MSI. In this study, both single and double bands of DNA can be clearly shown by silver staining. Silver staining is not only more sensitive than EB staining but also safer than isotope autoradiographs, and also dried gels can be preserved for years.

RER is a mark of genome-wide mutations and is usually described in malignant or premalignant tissue. RER^+ adenomas are more likely to undergo malignant transformation. Recent studies reported RER^+ phenotype in more than 80% of HNPCC and 15% of sporadic CRC. These RER^+ CRCs have the same biologic feature as HNPCC but are different from RER^- CRCs. The characteristics of RER+CRC are as follows: (1) early-onset, the highest ratio is occurred to patients below 35 years; (2) a predilection (70%) for the proximal colon (to the right of the splenic flexure), a higher rate of multiple CRC (89%) (intracolonic or extracolonic; synchronous or metachronous) than sporadic CRC; (3) DNA frequently diploid or near diploid; (4) primary resistance to some chemotherapy drug; (5) high mutation, manifold of the oncogene and/or the tumor suppressor gene (RAS, P53, APC et..); (6) abundant extracellular mucin, poor differentiation, exophytic growth patten, large size, a host Crohn's-like lymphocytic response to the tumor. Clinically RER^+ tumors have less metastasis and a relatively good prognosis.[7]

In our study, RER^+ was a predominant feature of poorly differentiated adenocarcinoma ($P<0.01$), and had a tendency to involve the right side of the colon ($P<0.05$), a higher proportion with family history ($P<0.05$), and Duckes, A and B stage ($P<0.05$). 4 HNPCC showed MSI at one or more loci, but none of them showed LOH. 75% (3/4) HNPCC tumors were demonstrated as RER^+. According to literatures RER^+ can be detected in about 15% of sporadic CRC, but in our studies 26.3% of sporadic cases were found to be RER^+. This difference could be caused by our case selection, which contained a high proportion of right-sided CRC and younger patients and small number of samples.

There might be two pathways leading to RER^+: One is chronic inflammation. Ulcerative colitis (UC) is considered to be a precancerous lesion. Brentnall found[8] that colorectal adenocarcinoma developed from UC often display RER^+. But the negative family history and its DNA repair mechanism was correct. At the molecular level, inflammation produces large quantities of reactive oxygen species, including hydroxy radicals (-OH), hydrogen peroxide ($H_2O_2^-$), and superoxide radicals (O_2^-). In the presence of reactive oxygen species, not only is DNA directly damaged, but also such conditions may result in a conformational change in the DNA template that prevents accurate replication by DNA polymerases. As a result, RER might have occurred during the chronic inflammatary process. Another pathway might be mismatch repair (MMR) gene mutation. Somatic mutation of two allelic of MMR genes (sporadic cancer) or one of MMR germ-line mutation accompanying another allelic gene somatic mutation (inheredic disorder) may be a response to the development of the cancer. MMR gene mutation may play an important role in tumorigenesis through the following mechanism: (1) Mutated MMR gene could enhance the frequency of oncogene and/or the suppressor gene mutation; (2) Germ-line mutated MMR gene may cause inherited genetic instability of some critical functional gene; (3) Hamper ability to repair DNA damaged by some chemical mutagens (such as alkylation agent) and the damage also can enhance carcinogenesis. Because of resistance of alkylation agent, the cell with MMR mutation grows abundantly to favor the development of tumors.[9]

The pathogenesis of CRC can be illuminated by the investigation of the relationship between RER and colorectal carcinogenesis. The detection of RER^+ make pre-symptomatic diagnoses of susceptibility to colorectal cancer become possible in HNPCC family members and will enable more effective surveillance programs for the early detection and treatment of this cancer. But there are still some questions that remain to be answered. For example, there is no unified criterion for RER state,and the number of MS loci and specific loci needed for MSI detecting to determine the RER state is unavailable, as a result, significant diversity of RER^+ ratio from different groups was reported. And also the mechanism of obtaining biological behavior of RER^+ CRC remains to be answered.

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