RESEARCH COMMUNICATION

Frequent MSI Mononucleotide Markers for Diagnosis of Hereditary Nonpolyposis Colorectal Cancer

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Abstract

Background: Failure in the DNA mismatch repair system is commonly accompanied by microsatellite instability and leads to colorectal cancer. The aim of this study was to find the most frequent of five mononucleotide markers in order to devise the simplest diagnostic strategy for identification of patients with hereditary nonpolyposis colorectal cancer (HNPCC) who were defined by defects in mismatch repair system. Materials and Methods: 78 patients with colorectal cancer were recruited for this investigation. Five mononucleotide markers, NR-27, NR-21, NR-24, BAT-25 and BAT-26, were used as a pentaplex panel to determine MSI status. Results: Two out of five mononucleotide markers, NR-21 (25.6%) and BAT-25 (23.1%) showed more instability than the others. Conclusion: In defining individuals with colorectal cancer, BAT25 and NR-21 may provide diagnostic assistance.

Keywords: Colorectal cancer HNPCC - MSI - mono nucleotide marker

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Introduction

Microsatellite instability (MSI) is genomic instability of simple repeated sequences. Microsatellites consist of stretches of repeating units of 1-5 base pairs (bp) that are distributed throughout the genome. (Thibodeau et al., 1993)

MSI is a mutational signature and the hallmark of colorectal cancer (CRCs) that has evolved as a result of inactivation of the DNA mismatch repair (MMR) system. (Boland et al., 2007). Approximately 3% of all CRCs are a consequence of Lynch Syndrome or hereditary nonpolyposis colorectal cancer (HNPCC), and nearly all the cases show MSI (Boland et al., 1997; 2005).

CRC is the second leading cause of cancer-related deaths in the Western world. There are 50,000 deaths due to the disease per year in the U.S (Greenlee et al., 2000).

According to Iranian Annual National Cancer Registration Report, colorectal cancer is the third most common cancer in women and the fifth among Iranian men. Incidence of colorectal cancer has been increased over the last three decades in Iran (Montazer Haghighi. et al., 2009).

Identification of the MSI phenotype is both promising and interesting as a screening tool to determine colorectal cancer. It is thought MSI can play an important role in the analysis of hereditary colon cancers. Thereby, it is commonly used as the first diagnostic screening test and step in the evaluation of an individual or family suspected of HNPCC (Loukola et al., 2001).

Since reference Bethesda panel has limitations resulting from the inclusion of dinucleotide markers, which are both less sensitive and specific for detection of tumors with MMR deficiencies (Agostini et al., 2010), we used a pentaplex PCR assay including five mononucleotide markers for a rapid and proper classification of MSI-H , MSI-L and MSS colorectal cancers (Buhard et al., 2004; Montazer Haghighi et al., 2009).

We performed this work to evaluate the possible benefit of using one quasimonomorphic mononucleotide repeat alone, as compared with using five mono nucleotide markers as a pentaplex, to identify HNPCC patients.

Materials and Methods

78 patients with HNPCC were chosen from 2008-2009. During these years they were referred and underwent colonoscopy at the Taleghani Hospital. Colonoscopy results were confirmed by our pathology department.

Patients who had inflammatory bowel diseases (IBD) or familial adenomatus polyposis (FAP) were excluded. Segments of genomic DNA at five sites, including known microsatellite sequences were amplified by polymerase chain reaction (PCR). Status of MSI in the 78 patients were examined using the pentaplex panel consisting of BAT-26, NR-21 BAT-25, NR-27 and NR-24 quasimonomorphic mononucleotide repeat. This panel, was first suggested by Buhard et al (2004).

Paraffin sections of normal and tumor tissue from colorectal cancer patients were examined for MSI status.

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Table 1. Primer Sequences for MSI Assay

Name	Gene	Genbank Number	Repeat	Primer Sequences	Amplicon Size (bp)
NR-27	Inhibitor of apoptosis protein-1	AF070674	27 A 5'UTR	F: AACCATGCTTGCAAACCACT R:CGATAATACTAGCAATGACC	87
NR-21	SLC7A8	XM 033393	21 T 5'UTR	F: GAGTCGCTGGCACAGTTCTA R: CTGGTCACTCGCGTTTACAA	109
NR-24	Zinc finger 2	X60152	24 T 3'UTR	F: GCTGAATTTTACCTCCTGAC R: ATTGTGCCATTGCATTCCAA	131
BAT-25	c-kit	X06182	25 T intron 16	F: TACCAGGTGGCAAAGGGCA R: TCTGCATTTTAACTATGGCTC	153
BAT-26	hMSH2	U04045	26 A intron 5	F: CTGCGGTAATCAAGTTTTTAG R:AACCATTCAACATTTTTAACCC	183

Primer sequences are presented in Table 1. The primers used for amplification of microsatellite sequences in this panel were those used previously (Buhard et al., 2004).

MSI status was determined by PCR of genomic DNA isolated from formalin-fixed, paraffin-embedded normal and tumor tissues from each patient. Tissue sections were deparaffinized in xylene, digested with proteinase K (2 mg/mL) overnight at 55°C, and DNA was isolated using DNAzol reagent (Life Technologies, Carlsbad, CA). The 5' anti-sense primer was labeled with a fluorescent dye using FAM for BAT-26 and NR-21, NED for BAT-25 and NR-27 and VIC for NR-24 (Buhard et al., 2004)

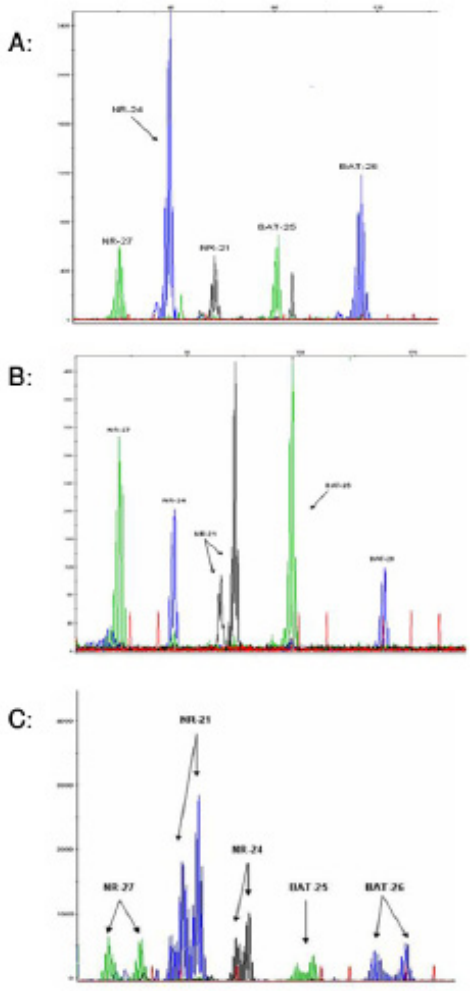

Figure 1. MSS Patterns. A: none of the five markers show instability) B: MSI-L (only NR-21 shows instability) C: (five out of five markers show instability)

(Montazer Haghighi et al., 2009) (Suraweera et al., 2002) (Kurzawski et al., 2004). PCR was carried out in a total volume of 25μl using a final concentration of 200μmol/L deoxyribonucleotide triphosphates (MBI Fermentas, St. Leon-Rot, Germany), 500 nmol/L each sense and antisense primer (Eurogentec, Seraing, Belgium), 1X PCR buffer (60 mmol/L Tris-SO4, pH 8.9; 18 mmol/L NH4 SO4; 2 mmol/L MgSO4). The five markers were synchronously amplified in a multiplex PCR. Denaturation at 95˚C for 5 minutes, 35 cycles of denaturation at 95˚C for 30 seconds, annealing at 55˚C for 30 seconds and extension at 72˚C for 5 minutes .Fluorescent PCR products were analyzed by capillary electrophoresis using an ABI 3130xl Genetic Analyzer (Applied Biosystems) and Genemapper software 3.7.

Tumors with instability at two or more of these markers were defined as being MSI-H, while those with instability at one repeat or showing no instability were determined as MSI-L and MSS tumors respectively.

The t-test was used to compare clinical features, pathology, or family history of individual with frequency of MSI markers. All P-values were two-sided; P<0.05 was considered statistically significant.

Results

Of the 78 patients analyzed with the pentaplex panel of mononucleotide repeats in the current study, 21patinets (26.9%) had tumors that were MSI-H, 11 patients (14.1%) were MSI-L and 46 patients (59%) were MSS. In the complete series with amplification of all five markers , the percentage of variant alleles were different for the markers, NR-21 and BAT-25, showed instability of 25.6 % and 23.1% (MSI-H and MSI-L) tumors, respectively (Figure 1). There were no statistically significant differences between the (MSI-H, MSI-L and MSS) regarding clinical features, pathology, or family history of cancer in the patients P> 0.05 (data not shown).

Discussion

Individuals with HNPCC inherit a mutation in one of the MMR genes which leads to MSI which can be observed in more than 90% Lynch syndrome tumors. Such genetic defects result in alteration in the size of microsatellite, and for this reason MSI can be applied as a phenotypic marker of incorrect DNA repair (Leite et al., 2010).

We evaluated alternative loci as the most frequent markers for detection of tumors with defects in MMR and the identification of a smaller optimal panel of markers for high instability (MSI-H) tumor detection. Assessment of MSI status is used for the diagnosis of HNPCC. As a result of an increasing demand for this molecular test, it is very important to arrange rapid and cost-effective tests with panels of microsatellite markers able to satisfy both clinical requirements.

We determined that BAT-25 and NR-21 were more frequent mononucleotide markers and may be useful for early detection of individuals with HNPCC.

In accordance with previous study by Leite et al (Leite et al., 2010) that they reported the highest frequency of MSI 27.3% was exhibited by the BAT-25 marker. Furthermore they revealed that the BAT-25 was the most sensitive marker (86.7%) as well.

Nevertheless, Xicola et al (Xicola et al., 2007) compared National Cancer Institute (NCI) panel which is included 3 dinucleotid markers, BAT25 and BAT26 compare with pentaplex panel including five mononucleotide repeats (BAT-26, BAT-25, NR-21, NR-22, and NR-24). They showed that the pentaplex panel of mononucleotide repeats performs better than the NCI panel is detection of mismatch repair-deficient tumors. Simultaneously their assessment showed that the instability of BAT-26 and NR-24 is as frequent as the use of the pentaplex panel for diagnosing mismatch repair deficiency.

More recently, Buhard et al., (2006) evaluated the incidence of polymorphisms in different populations around the world including polymorphisms of four of the five mononucleotide markers originally suggested by Suraweera et al., (2002) as well as those of NR-27. They found that BAT-25 and BAT-26 were polymorphic to a statistically significant degree only in African populations (up to 15% of affected individuals). NR24 was found to be monomorphic in almost all individuals of the worldwide series, and there were substantial percentages of polymorphisms in NR-21 in Africa and Oceania (Buhard et al., 2006).

Polymorphisms in NR-21 was reported previously from Central/South Asia (Makrani from Pakistan) and two Chinese minorities from East Asia (Dai and Tujia) (Buhard et al., 2006). We confirm here a relatively high incidence of polymorphism in NR-21 in our population.

Although the findings of our investigation confirmed the results of BAT-25 marker reported by Leite et al., (2010) our results are different from the reported by Xicola et al (2007). Hence it seems that any population has its frequent marker to present and consider as a smaller panel for rapid and early detection of colorectal cancer. So it would be logical that in order to approach a rapid detection in every population firstly the most frequent markers should be consider and if they will not able to show MSI status then the others markers will be used. It would be notable that the frequency of polymorphism for each marker is highly variable from one population to another. Finally, our findings bear important consequences for the clinical setting and screening colorectal caner in Iranian population. However, an investigation suggested that with a larger sample size and a different ethnic group would be more informative.

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