TP53 Codon 72 Heterozygosity May Promote Microsatellite Instability in Sporadic Colorectal Cancer

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Received: 5/ Jan/2009, Accepted: 17/ Oct/2009

Abstract

Objective: The polymorphic variants at codon 72 of the p53 gene, encoding proline or arginine at residue 72, produce marked changes in the p53 structure. From the evidence that the DNA mismatch repair system and p53 interact to maintain genomic integrity, we hypothesized that codon 72 variations may influence the prevalence of microsatellite instability (MSI), a feature of malignancies associated with mismatch repair deficiency in sporadic colorectal cancer.

Materials and Methods: We investigated the frequency of MSI in three P53 codon 72 genotypes using genomic DNAs from 144 paraffin blocks of sporadic colorectal adenocarcinomas by testing the BAT-26 poly(A) marker. We used PCR-SSCP analysis to detect tumor sample MSI for the nonisotopic detection of deletions in the BAT-26 poly (A) mononucleotide repeat. Associations between qualitative variables were evaluated using the $\chi^2$-test. Statistical significance level was set to $p \leq 0.05$.

Results: MSI analysis revealed that 24.3% of the tumors ($n=35$) were MSI-positive and 75.7% ($n=109$) were MSI-negative. The frequency of microsatellite instability in the arginine/arginine, arginine/proline and proline/proline genotypes were 11 (16.9%), 22 (36.1%) and 2 (11.1%) respectively. A significant difference in distribution of MSI was found for the arginine/proline genotype compared with the grouped arginine/arginine and proline/proline genotypes ($p=0.05$).

Conclusion: Our findings suggested that colorectal adenocarcinomas arising in individuals with the p53 codon 72 arginine/proline heterozygosity are more prone to microsatellite instability than those with other p53 genotypes. In our study, MSI was important in the carcinogenesis of sporadic colorectal cancer arising in pro/arg heterozygotes.

Keywords: Microsatellite Instability, Polymorphism, p53, Colorectal Neoplasms

Introduction

Two different genetic pathways have been involved in tumorigenesis. The cause of approximately 85% of all colorectal cancers (CRC) is chromosomal instability microsatellite stable (MSS) carcinomas characterized by alterations in oncogenes and tumor suppressor genes. These cancers frequently show gross genomic instability which accounts for the initiation and progression events leading to transformation of normal colonic epithelial cells to carcinoma. In 10–15% of all CRC cases, microsatellite instability (MSI) is involved, resulting from alterations in the DNA repair genes or mismatch repair (MMR) system responsible for repair of errors occurring during DNA replication (1-5). Microsatellites are mononucleotide or dinucleotide DNA repeat sequences present throughout a genome. Instability is characterized by mostly single base-pair insertions or deletions in these repeat loci causing widespread genomic instability (4).

The TP53 gene, located at chromosome 17p13, is one of the most mutated genes causing many types of human cancers (6-9). In addition to mutations, several polymorphisms in the wild-type p53 gene locus have been detected which could alter its function (8,10). The most common polymorphism associated with cancer development in the general population is the codon 72 arginine (Arg)
TP53 Codon 72 Polymorphism and Microsatellite Instability

Materials and Methods

Study population and samples
We have detected p53 codon 72 polymorphism in the genomic DNA of 180 paraffin blocks of sporadic colorectal adenocarcinoma cases at Alzahra Hospital (Isfahan) over the period between 2002 and 2006 (14). Because of the genomic DNA unsuitability of 36 specimens for MSI testing, in this study we performed a microsatellite instability study on the genomic DNA of 144 patients from the 180 mentioned colorectal adenocarcinoma patients’ paraffin blocks. DNA obtained from each specimen’s adjacent cancer-free colonic tissue was used as control. Before commencing the study, approval was granted by the Isfahan University of Medical Sciences’ Research Ethics Committee.

PCR-SSCP of the BAT-26 poly (A) Tract for detection of MSI
It was previously determined that BAT-26 analysis is sufficient to establish tumor MSI status (36). BAT-26, a poly (A) tract localized in intron 5 of MSH2, neither showed significant size variations between both alleles nor between individuals in DNA from normal tissues and colorectal tumors and cell lines, and was thus quasi-monomorphic (36). Therefore, in order to explore a possible association between the presence of MSI with different genotypes at codon 72 of the TP53 tumor suppressor gene, we performed a nonsotopic detection of deletions in the BAT-26 poly (A) mononucleotide repeat; a polymerase chain reaction single-strand conformation polymorphism (PCR-SSCP) analysis as previously described (36, 37) was used for MSI detection in the tumor samples already tested for determination of p53 codon 72 polymorphism in their genomic DNAs.

In the MSI detection analysis, primers were designed according to the procedure described by Zhou et al. (36). Primer sequences used for amplification of the BAT-26 mononucleotide repeat were as follows:

Forward 5’ TGACTACCTTTTGACTTCAGCC 3’
Reverse 5’ AACATCCAACATTTTAAAAACC 3’

PCR conditions comprised of a 5 minute denaturation at 94°C, a 2 minute annealing at 45°C, a 2 minute extension at 70°C, followed by 32 cycles of 1 minute annealing at 45°C, 1 minute extension at 70°C and 30 seconds of denaturation at 94°C. The program was terminated with 5 minutes of extension at 70°C. The PCR product was heat-denatured into single-strand DNA for 5 minutes at 94°C in a formamide loading buffer (95% formamide, 10 mM EDTA, 0.05% bromophenol blue, 0.05% xylene cyanol), then separated on a mini-gel electrophoresis apparatus (Farayand Danesh Arjan, Iran) using a non-denaturing 15% acrylamide gel containing 5% glycerol (60:1 acrylamide:bis-acrylamide). Gel samples were run at ambient temperature, and without cooling. Gels were run for 2.5 hours at 90 V. Silver staining was carried out as previously described (38). The presence of additional, faster migrating bands compared with normal tissue DNA were indicative of the presence of a shortened BAT-26 poly (A) tract and therefore of the MSI-positive phenotype (36).

Statistical analyses
Associations between qualitative variables were evaluated using the $\chi^2$ test. Statistical significance level was set at $p \leq 0.05$.

Results
Our analysis was performed on 144 samples of sporadic colorectal adenocarcinoma and their adjacent normal colonic tissue. The ages of 144 patients (55 women and 89 men) ranged from 38 to 90 years. Mean age was 58.4 ± 11.3 years...
in MSI-positive and 66.6 ± 12.1 years in MSI-negative patients (p = 0.532). Detection of TP53 codon 72 polymorphism by allele specific PCR had been conducted in all colorectal adenocarcinoma specimens (14). From 144 specimens, 45.1% were Arg/Arg, 42.4% were Arg/Pro and 12.5% were Pro/Pro (Table 1).

Allelic frequencies were 0.663 for the arginine allele and 0.337 for the proline allele. The MSI study was performed on 144 patients using size variation analysis within the BAT-26 poly (A) tract (36). Silver stain PCR-SSCP analysis showed a specific PCR product of 121 bp. Cases showing an unequivocally distinct additional band or shifts in the tumor tissue DNA compared with normal tissue DNA were recorded and classified as MSI-positive (Fig 1). MSI analysis revealed that 24.3% of the tumors (n=35) were MSI-positive and 75.7% (n=109) showed no change and were MSI-negative. MSI was more frequently observed in tumors arising in the Arg/Pro genotype (Table 1).

A significant difference in distribution of MSI was found in the Arg/Pro genotype compared with the (grouped) Arg/Arg and Pro/Pro genotypes (p=0.05).

The association of MSI with various clinicopathological parameters of colorectal cancer cases is shown in Table 2.

Table 1: Distribution of MSI in different TP53 codon 72 genotypes among colorectal cancer cases

<table>
<thead>
<tr>
<th>Genotype</th>
<th>N (%)</th>
<th>MSI-positive</th>
<th>MSI-negative</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (%)</td>
<td>Positive</td>
<td>Negative</td>
<td></td>
</tr>
<tr>
<td>A/P</td>
<td>61 (42.4)</td>
<td>22 (36.1)</td>
<td>39 (63.9)</td>
<td>0.05</td>
</tr>
<tr>
<td>A/A</td>
<td>65 (45.1)</td>
<td>11 (16.9)</td>
<td>54 (83.1)</td>
<td></td>
</tr>
<tr>
<td>P/P</td>
<td>18 (12.5)</td>
<td>2 (11.1)</td>
<td>16 (88.9)</td>
<td></td>
</tr>
</tbody>
</table>

A/A: Arg/Arg genotype
A/P: Arg/Pro genotype
P/P: Pro/Pro genotype
N: number
MSI: Microsatellite Instability

Chi-square analysis revealed significant associations between MSI presence of MSI and the proximal location of tumors (p=0.028), Dukes colon cancer stages A-B (p<0.001), mucinous adenocarcinoma (p=0.019) and well differentiated adenocarcinomas (p=0.023). No correlation was observed between MSI and gender or age of the patients.

Table 2: Colorectal cancer patient general and clinicopathologic data

<table>
<thead>
<tr>
<th>Parameters</th>
<th>N (%)</th>
<th>MSI-positive N (%)</th>
<th>MSI-negative N (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>144</td>
<td>35 (24.3)</td>
<td>109 (75.7)</td>
<td>0.883</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>89</td>
<td>22 (24.7)</td>
<td>67 (75.3)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>55</td>
<td>13 (23.6)</td>
<td>42 (76.4)</td>
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<tr>
<td>Location</td>
<td></td>
<td></td>
<td></td>
<td>0.028</td>
</tr>
<tr>
<td>Proximal</td>
<td>93</td>
<td>28 (30.1)</td>
<td>65 (69.9)</td>
<td></td>
</tr>
<tr>
<td>Distal</td>
<td>51</td>
<td>7 (13.7)</td>
<td>44 (86.3)</td>
<td></td>
</tr>
<tr>
<td>Duke’s stage</td>
<td></td>
<td></td>
<td></td>
<td>0.000</td>
</tr>
<tr>
<td>A-B</td>
<td>36</td>
<td>21 (58.3)</td>
<td>15 (41.7)</td>
<td></td>
</tr>
<tr>
<td>C-D</td>
<td>108</td>
<td>14 (13.0)</td>
<td>94 (87.0)</td>
<td></td>
</tr>
<tr>
<td>Tumor type</td>
<td></td>
<td></td>
<td></td>
<td>0.019</td>
</tr>
<tr>
<td>Mucinous adenocarcinoma</td>
<td>23</td>
<td>10 (43.5)</td>
<td>13 (56.5)</td>
<td></td>
</tr>
<tr>
<td>Nonmucinous adenocarcinoma</td>
<td>121</td>
<td>25 (20.7)</td>
<td>96 (79.3)</td>
<td></td>
</tr>
<tr>
<td>Tumor grade</td>
<td></td>
<td></td>
<td></td>
<td>0.023</td>
</tr>
<tr>
<td>Well</td>
<td>92</td>
<td>28 (30.4)</td>
<td>64 (69.6)</td>
<td></td>
</tr>
<tr>
<td>Moderate-Poor</td>
<td>52</td>
<td>7 (13.5)</td>
<td>45 (86.5)</td>
<td></td>
</tr>
</tbody>
</table>

MSI: Microsatellite instability
TP53 Codon 72 Polymorphism and Microsatellite Instability

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Fig 1: Identification of the MSI phenotype in colorectal cancer using silver stain PCR-SSCP screening for deletions in the BAT-26 mononucleotide repeat. A PCR product of 121 bp was obtained. Patient numbers are designated above each block. An additional aberrantly migrating band (arrow) compared to the adjacent normal tissue (N3) is clearly visible in cancer specimen number 3 (C3), indicating presence of the MSI phenotype in this tumor. Other tumor samples contain normal BAT-26 banding profile.

Discussion

Mutation-caused MSIs, such as those with insertions or deletions in microsatellite repeats, are involved in the genesis of about 15% of sporadic CRCs and most of hereditary nonpolyposis colorectal cancers (HNPPC) (1-3). Multiple errors in repetitive DNA sequences (microsatellites) result from a failure of the MMR system to edit errors made during DNA replication (4). MMR genes play a major role in the correction of DNA damage. Mutations in cell cycle control and MMR genes disable cell damage repair caused by genetic alterations. Loss of MMR proteins appears to be an important event in the carcinogenesis process or in the primary stages of tumor progression in certain cancer types (39). A defective MMR system increases replicative error rates throughout the cancer-cell genome, and there is evidence that some tumors with apparent MMR defects may also have MSI (40). Also, loss of MMR may result in loss of cell cycle control and/or resistance to apoptosis, both of which promote neoplastic formation (41). Therefore, detection of MSI, a functional marker of MMR defects, might be useful for detection. Detection the MMR system is inactivated either by promoter hypermethylation, or germ-like mutations in MMR genes (2-4). Different markers are used for MSI detection. In most MSI positive tumors, the 26 deoxyadenosines in the BAT-26 poly (A) tract within the intron 5 of MSH2 gene are typically shortened in length by 4–16 bp (42). It has been shown that silver-stained SSCP gels only 8 cm in length can readily detect deletions of as little as 3 bp in BAT-26. Together with the observation that screening for BAT-26 deletion is more than 99% accurate for MSI status identification (36), the reported frequency of BAT-26 polymorphisms in pure Caucasian populations (if you mean white people, it's more precise to say 'populations of European descent') is sufficiently low (0.08%) (43, 44). For false positives not to be a concern in the routine analysis of MSI, BAT-26 appears to undergo significant deletions in the large majority of tumors with MSI, as demonstrated by comparison with alterations to several different microsatellite loci. The hypersusceptibility of BAT-26 to deletion in tumors with the MSI phenotype obviates the need to examine a panel of five different microsatellite loci as was proposed by Zhou et al. (36).

Colorectal carcinogenesis is a complex multistage process that shows a high frequency of p53 alterations and the majority of its resulting cancers are adenocarcinomas (45, 46). Exon 4 of TP53 is one of this gene's largest exons, lies close to the central region, and is important for DNA-specific binding (47). A common p53 polymorphism, encoding either proline or arginine at residue 72, produces characteristic changes in the structure of p53. This polymorphism also lies in a part of p53 involved in apoptosis induction (47). Because of functional differences between the two polymorphic variants of p53, genotype at codon 72 may affect susceptibility to cancer development. The p53 codon 72 polymorphism has been controversially associated with higher risk of developing various human cancers such as colorectal (14, 48-59), lung (15), esophageal (16), cervical (17), bladder (18), breast (19, 20), head and neck (21), pancreas (22), nasopharynx (23) and liver (24) cancers. The role of codon 72 polymorphism of the p53 gene had been noted in colorectal cancer patients of many populations including those in Iran (14), Argentina (48), Taiwan (49), Spain (50), China (51), Japan (52-54), Turkey (55), Germany (56), France (57), Sweden (58) and the USA (59). The study of this polymorphism in different racial populations has shown conflicting results. It has also been shown that codon 72 polymorphism varies greatly in different ethnic populations (60) and these ethnic differences might have a significant effect on cancer risk in different ethnic populations.

In this study we showed a significant association between the p53 codon 72 variations and the presence of MSI in colorectal cancer patients. MSI was more frequently observed in tumors arising in the arginine/proline genotype. These findings support the notion of cooperation between p53 and the MMR system and indicate that heterozygosity for p53 codon 72 increases genomic instability at
the nucleotide level. Three possible explanations may account for the p53 dependent increase in MSI. First, heterozygosity of p53 codon 72 may reduce genomic instability at the nucleotide level by either directly or indirectly mediating repair. This is supported by the observation that p53 is capable of attaching to insertion/deletion loops, the DNA lesions associated with MSI (61). A second possibility is that the Arg72 allele is preferentially mutated and retained in various human tumors arising in Pro/Arg heterozygotes, and that the p53 mutant plays the role of a more potent inhibitor of p73, which is a member of the p53 family and has an apoptotic function when p53 contains Arg72 rather than Pro72 (62). These findings suggest that this p53 polymorphism acts as an intragenic modifier of the protein’s mutant behavior and has an effect on its biological role. A third possibility is that p53 heterozygotes may lead to the upregulation of MMR defects. In this study, we did not consider MMR defects in relation to p53 genotypes and/or MSI detection, but now acknowledge its importance in future studies.

In our study, MSI was important in the carcinogenesis of sporadic colorectal cancer arising in Pro/Arg heterozygotes. There are rare studies on MSI and p53 codon 72 polymorphism. In accordance with our results, Sobczuk et al (63) showed that MSI seems to be important in the development of sporadic endometrial cancer in Poland (p<0.05). In contrast to our finding, other studies did not show significant association between MSI and predisposition to thyroid (64), breast (65), gallbladder (66) and bladder (67) cancers. Furthermore, we observed that MSI is related to well differentiated tumors which elucidates that tumors developing through the MSI genetic pathway are less aggressive. Also, we observed that the mucinous histology and proximal location of tumor are related to the presence of MSI. These results are consistent with another study (68) showing that mucinous histology is related to proximal tumors possessing the MSI phenotype. Also, our results are consistent with those of another research in Iran (69) showing that proximal tumor location is related to MSI presence. Although our study was not controlled for p53 mutations, our results confirmed their inverse relationship with MSI in Iranian colorectal cancer specimens. This is an important matter to address in further studies in order to assess the role of p53 alterations and MSI in colorectal cancer specimens.

MSS colorectal cancers exhibit more frequent somatic alterations in p53 with loss of function than highly unstable microsatellite cancers (70). Thus, inherited variants in p53, such as the codon 72 polymorphism, would have a minor impact in patients with MSS tumors. MMR insufficient cells may also be more dependent on the p53-mediated apoptotic pathway, as the MMR system itself seems to play a potentially apoptotic role in a largely p53-independent manner (71).

**Conclusion**

At present, significance of the p53 codon 72 polymorphism remains obscure, both in terms of cancer epidemiology, and pathobiology. Additional comprehensive studies using a spectrum of excised human carcinoma tissues from more numerous tumor samples will be needed to elucidate the association between polymorphic residue within p53 and the microsatellite behavior of MMR in human carcinogenesis.

**Acknowledgments**

This work was supported by grant number 184125 provided by Isfahan University of Medical Sciences’ research director. We are grateful to the participants from the biotechnology department of the School of Pharmacy at Isfahan University of Medical Sciences and to Ms. Fatemeh Moazzen for her technical assistance. There is no conflict of interest in this article.

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