p53 is a tumor suppressor gene that encodes a 53 kd nuclear phosphoprotein which appears to function as a negative regulator of cell proliferation. The p53 tumor suppressor gene mutates in diverse types of human cancer. The mutations of the p53, and the loss of genetic material from chromosome 17p is associated with tumors. The loss of heterozygosity is measured by the use of informative restriction fragment length polymorphisms (RFLPs). There are several RFLP's described within the p53 gene, either intronic or exonic[1-7]. We previously reported data on the polymorphism of p53 on exon 6 (CD 213) in a healthy Turkish population, and in Turks with different types of tumors. However, this site was found to be very rare[8].

The aim of this study was to screen three different RFLP’s (CD 47, CD 213, Int 6) in healthy subjects from Turkey in order to find an informative site.

MATERIALS and METHODS

Four different RFLPs were studied according to previously described techniques with the use of polymerase chain reaction (PCR) of exon 4, exon 6 and intron 6. DNA was extracted by conventional methods.

Exon 5 CD 47 (Proline-Serine C-T) polymorphism was studied according to previously described techniques with modification[7]. DNA samples were subjected to PCR in the presence of primers 5'GCACTGACCGTGAAGTCA3' and 5'ATC-
TACAGTCCCCCTTGCCG-3'. PCR was carried out by 35 cycles under the following conditions: 1 min at 95°C; 1 min at 60°C and 2 min at 72°C.

The resulting PCR products (296 bp) were digested with NciI (Promega, Madison MI, USA), at 37°C and analyzed by 2% agarose gel electrophoresis. NciI recognizes the proline[7] (Figure 1).

Exon 6 CD 213 (A-G) polymorphism was studied according to previously reported techniques[8].

Intron 6 (A-G) alteration, which is localized 61 bp downstream of exon 6 of the p53 gene, was studied with the previously described technique using the enzyme MspI (Biolabs, MA, USA), analyzed at 37°C, then by 1% agarose gel electrophoresis[5] (Figure 2).

RESULTS and DISCUSSION

Mutation or loss of heterozygosity of p53 results in the loss of the tumor suppressor function, and this has been associated with tumor progression. The state of "loss of heterozygosity" can be studied by the informative restriction fragment length polymorphisms (RFLPs). There exist several RFLPs at the p53 gene locus. The aim of this study was to analyze four p53 polymorphisms in normal Turkish individuals. These polymorphisms are located at exon 4 (CD 47), at exon 6 (CD 213) and intron 6 (A-G), as previously reported[5-7].

The results of the RFLP's in a healthy Turkish population are shown in Table 1. CD 47 (C-T) polymorphism is localized in the transactivation domain of the p53 gene. Felley Bosca et al. reported the frequency of the rare allele to be 0% in Caucasians (n: 69) and 4.7% among African-Americans[8].

<table>
<thead>
<tr>
<th>Number of chromosomes</th>
<th>Normal allele</th>
<th>Gene frequency (%)</th>
<th>Heterozygosity (n) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD 47 (pro)</td>
<td>102</td>
<td>1.0</td>
<td>0</td>
</tr>
<tr>
<td>CD 213 (A)</td>
<td>88</td>
<td>0.9886</td>
<td>1</td>
</tr>
<tr>
<td>Int 6 (G)</td>
<td>110</td>
<td>0.70</td>
<td>18</td>
</tr>
</tbody>
</table>

Table 1. The results of RFLPs in a healthy Turkish population

Figure 1. Direct detection of p53 Exon 4 CD 47 (C-T) polymorphism with Nci I Restriction enzyme analysis (agarase gel electrophoresis, 2%) Lane 1: ØX174 Hae III (marker) Lane 2 and 7: Uncut PCR product (296 bp) Lane 3-6: PCR products cut with Nci (254 bp)

Figure 2. Direct detection of p53 Intron 6 (A-G) polymorphism with Msp I Restriction enzyme analysis (agarase gel electrophoresis, 2%) Lane 1: ØX174 Hae III (marker) Lane 2: Uncut PCR product (351 bp) Lane 3-5,9,11: Homozygote for G-G (221 bp). (+/+) Lane 6: Homozygote for A-A (351 bp) (-/-) Lane 7,8,10: Heterozygote individual G and A (351 and 211 bp). (1/-)
Our data regarding the CD 47 was consistent with their results of Caucasians. The polymorphic A/G nucleotide is localized in intron 6, 61 bp downstream of exon 6 of the p53 gene. The polymorphic "G" site was reported to be 0.74 and 0.69\textsuperscript{[5,9,10]}. The polymorphic A site is very rare in Chinese (0.054, 0.024); in Indians (0.185) and in African Blacks (0.186)\textsuperscript{[11]}. Our results were consistent with the previously described data for Caucasians. The frequency of the "G" site and the observed heterozygosity were 0.70 and 32.72, respectively.

Furthermore, we had previously studied the rare polymorphism at exon 6 (CD 213) of the p53 gene in the Turkish population\textsuperscript{[8]}. A-G alteration was only present in 2.27% of Turkish individuals. CD 213 is a hot spot mutation site for BL, and it was reported that 4, of the 13 BL's (30%), carried the polymorphism\textsuperscript{[12,13]}. Of the 16 Turkish BL samples, none of them had the A to G alteration (data not shown). This discrepancy needs to be investigated further in more BL samples.

Intraexonic polymorphisms that we have studied (CD47, CD213) were found to be very rare in the Turkish population. However, intron 6 A/G polymorphism can be used for a further analysis of the "loss of heterozygosity" in malignancies.

REFERENCES


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