Intronic SNPs of TP53 gene in chronic myeloid leukemia: Impact on drug response

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Abstract

Background: TP53, located on chromosome 17p13, is one of the most mutated genes affecting many types of human cancers. Thus, we aimed at investigating the association of SNPs in TP53 gene with chronic myeloid leukemia (CML). Materials and Methods: A total of 236 CML and 157 control samples were analysed for mutations in TP53 gene using polymerase chain reaction followed by direct sequencing. Results: Sequencing analysis for mutations in exons 7–9 of the TP53 gene revealed four SNPs, three in intron 7 (C14181T, T14201G, and C14310T) and one SNP in intron 6 (A13463G) of TP53 gene. The mutation C14181T is located at position 72 base pairs downstream of the 3′-end of exon 7 of the P53 gene. This mutation is in complete linkage disequilibrium with a T14201G mutation, 20 base pairs further downstream occurring at position 14201. This mutation occurred only in the presence of C14181T mutation and these mutations showed association with advanced phase and cytogenetic poor response. Another two novel mutations, C14310T in intron 7 and A13463G in intron 6 were also found to be associated with cytogenetic poor response. Conclusion: Our study suggests that TP53 intronic SNPs might have a strong influence on disease progression and poor response in CML patients.

Key words: Association, chronic myeloid leukemia, progression, response, single nucleotide polymorphism, TP53 gene

INTRODUCTION

TP53 gene, located on chromosome 17p13, consists of 11 exons coding for an mRNA of 2.2–2.5 kb and approximately 53 kDa protein of 393 amino acids. TP53 is the most important tumor suppressor gene that is involved in many pathways such as apoptosis, cellular transcriptional regulation, and cell cycle control.[1,2] Mutations in the p53 gene occur in approximately 50% of all human tumors.[3] It is one of the most mutated genes observed in many types of human cancers.[4,5] The majority of both sporadic and germline TP53 mutations found in cancer cells were missense point mutations, occurring mainly in the DNA binding domain of the protein.[6] P53 is a highly conserved gene and exons 5–8 contain four of the five evolutionarily conserved domains of p53 gene encoding amino acids 117–286. The majority of the reported mutations were found in this region.[7]

The frequency of p53 mutations in hematological malignancies were reported to be relatively low compared to other tumors, but the incidence was found to be increased in some cases with disease progression.[8,9] It is well known fact that p53 gene alterations might play a role in the progression of the chronic myeloid leukemia (CML), in fact close relation between myeloid blast crisis with loss of a 17p and p53 gene point mutations was reported[10] and 30% of the blast crisis patients were reported to carry p53 gene mutations.[11] P53 polymorphisms were also reported in the intronic regions, which are thought to have regulatory roles in gene expression leading to susceptibility to cancer.[12] Intronic variants might affect gene regulation through aberrant splicing or through disruption of critical DNA–
protein interactions.\textsuperscript{[13]} Previous studies reported that polymorphism in intron 6 was significantly associated with increased risk for several cancers such as GI (gastrointestinal) tumors, breast, Li–Fraumeni syndrome, thyroid, and ovarian.\textsuperscript{[14–17]} These reports suggest that intronic mutations affect the stability of the \textit{p53} protein and thereby its growth-suppression function. This study reports mutations in intron 6 and 7 of \textit{TP53} gene in CML patients.

**MATERIALS AND METHODS**

This study comprises of 236 CML cases reported at Nizam’s Institute of Medical Sciences, Hyderabad, during 2004–2006. A total of 157 age- and sex-matched healthy individuals without family history of cancer were selected to serve as a control group. Informed consent was taken from all the individuals recruited for the study, after obtaining ethical committee clearance. Five milliliter of blood samples were collected in EDTA vacutainers from both the CML patients and control groups. Patients clinical data like phase of the disease and cytogenetic response was noted from the tumor registry file with the help of oncologist. Response status was classified into major (1–35%), minor (35–65%), and poor (above 65%) based on the percentage of \textit{ph}+ve cells and duration of response to imatinib therapy. Genomic DNA was isolated by using the salting-out method\textsuperscript{[18]} and used for mutational analysis.

A 964 bp fragment was amplified using a set of primers, forward: 5’-CTG CTT GCC ACA GGT CTC-3’ and reverse: 5’-GAC AAT GGC TCC TGG TTG TA-3’ covers both exonic and intronic regions from exons 7 to 9. These exons were located in highly conserved domains of DNA binding region of \textit{TP53} gene, this region carries majority of mutations. Polymerase chain reaction (PCR) master mix (100 μl for 10 samples) contains template DNA-50 ng, PCR buffer-10 μl, MgCl₂-4 μl, dNTPs-5 μl, primer 2 μl of each (5 picomoles), taq Polymerase-3 U, MilliQ water-65 μl. PCR was carried out for 36 cycles with initial denaturation at 94 °C for 2 min followed by denaturation at 95 °C for 1 min, annealing at 58 °C for 45 s, extension at 72 °C for 2.30 min and final extension at 72 °C for 7 min. After PCR all the products were checked on 1.5% agarose gel for the presence of amplification. The amplified samples were subjected to direct sequencing (ABI Prism 3730 DNA Analyser).

**Statistical analysis**

The \( \chi^2 \)-test was used to assess the significance of any difference in the prevalence of \textit{TP53} polymorphisms between the CML patients and controls. Allele frequencies were calculated through the counting method. Odds ratio was estimated to calculate the relative risk for each genotype to develop disease. Statistical significance was taken as \( P<0.05 \).

**RESULTS AND DISCUSSION**

Intronic mutations are known to affect the stability of the \textit{p53} protein and thereby its growth-suppression functions. Sequencing of 7–9 exons of \textit{TP53} gene showed four SNPs, three in intron 7 and one in intron 6. The first C \( \rightarrow \) T SNP is located at position 14181, 72 base pairs downstream of the 3’-end of exon 7 of the \textit{P53} gene. This SNP is in complete linkage disequilibrium with a T \( \rightarrow \) G mutation, 20 base pairs further downstream occurring at position 14201.\textsuperscript{[19]} The T14201G SNP occurred at the enhancer site of intron but did not abolish it. This mutation occurred only in the presence of C14181T mutation [Figures 1 and 2]. All the samples with C \( \rightarrow \) T mutation had T \( \rightarrow \) G mutation indicating that these mutations belong to the same allelotype and might have resulted from the same mutational event. Previous findings on acute myeloid leukemia and urinary bladder cancer also showed no significant association between mutant haplotype in intron 7 and cancer development.\textsuperscript{[20]} With respect to age at onset and sex of the proband, we could not find association with mutant haplotype. Odds ratio was estimated to calculate the relative risk for each haplotype to develop CML, odds ratio could not revealed any association between the intron 7 haplotypes and disease group [Table 2].

The frequency of mutant haplotype (TT-GG) was found to be elevated in patients with advanced phase blast crisis (9.5%) compared to chronic (4.2%) and accelerated (5.3%) phase of the CML. With respect to drug (Imatinib) response status, poor cytogenetic responders (10.5%) showed elevated frequency of TT-GG mutant haplotype as compared to major (3.3%) and minor (3.8%) responders [Table 1]. The significant association of intron 7 polymorphism (TT-GG mutant haplotype) with advanced phase and poor cytogenetic response suggests that these intronic mutations might have strong influence on disease progression and drug response.

Two novel mutations were observed in our study, one in intron 6 (A13463G) and other in intron 7 (C14310T) one in...
Table 1: Distribution of TP53 gene polymorphisms C14181T/T14201G with epidemiological and clinical variables

| Parameters                                      | CCTT No (%) | CTTG No (%) | TTGG No (%) | Allele frequency |
|-------------------------------------------------|-------------|-------------|-------------|------------------|
| Cases (236)                                     | 170 (72.0)  | 55 (23.31)  | 11 (4.66)   | 0.84             |
| Controls (157)                                  | 104 (66.2)  | 39 (24.84)  | 14 (8.22)   | 0.79             |
|χ² = 3.23; df = 2, P = 0.199                     |             |             |             |                  |
| Age of the proband                              |             |             |             |                  |
| <20 years (23)                                  | 12 (52.2)   | 8 (34.8)    | 3 (13.0)    | 0.7              |
| 20–30 years (57)                                | 45 (78.9)   | 11 (19.3)   | 1 (1.8)     | 0.89             |
| 30–40 years (54)                                | 37 (68.5)   | 11 (20.4)   | 6 (11.1)    | 0.79             |
| >40 years (102)                                 | 76 (74.5)   | 25 (24.5)   | 1 (1.0)     | 0.87             |
|χ² = 16.058; df = 6, P = 0.013                    |             |             |             |                  |
| Sex of the proband                              |             |             |             |                  |
| Males (161)                                     | 117 (72.7)  | 37 (23.0)   | 7 (4.3)     | 0.84             |
| Females (75)                                    | 53 (70.7)   | 18 (24.0)   | 4 (5.3)     | 0.83             |
|χ² = 0.158; df = 2, P = 0.925                    |             |             |             |                  |
| Clinical phase of CML                           |             |             |             |                  |
| Chronic (189)                                   | 137 (72.5)  | 44 (23.3)   | 8 (4.2)     | 0.84             |
| Accelerated (19)                                | 13 (68.4)   | 5 (26.3)    | 1 (5.3)     | 0.82             |
| Blast Crisis (21)                               | 16 (76.2)   | 3 (14.3)    | 2 (9.5)     | 0.83             |
|χ² = 1.986; df = 4, P = 0.738                    |             |             |             |                  |
| Cytogenetic response                            |             |             |             |                  |
| Major (123)                                     | 87 (70.7)   | 32 (26.0)   | 4 (3.3)     | 0.84             |
| Minor (26)                                      | 22 (84.6)   | 3 (11.5)    | 1 (3.8)     | 0.9              |
| Poor (38)                                       | 27 (71.1)   | 7 (18.4)    | 4 (10.5)    | 0.8              |
|χ² = 6.19; df = 4, P = 0.185                    |             |             |             |                  |

Table 2: Distribution of odds ratio

| Odds ratio (95% CI) | Cases/controls | Sex of the proband |
|---------------------|----------------|--------------------|
| CCTT/CTTG           | 0.896 (0.535–1.390) | 0.931 (0.486–1.783) |
| CCTT/TTGG           | 0.480 (0.210–1.098) | 0.792 (0.222–2.824) |
| CTGG/TTGG           | 0.557 (0.228–1.356) | 0.851 (0.220–3.288) |

CONCLUSIONS

TP53 mutations in intronic region may initiate aberrant...
Intron variants of the p53 gene are associated with increased premessenger mRNA splicing, producing an mRNA that may be translated into defective p53 protein which increases the likelihood of a deleterious phenotype that inhibits the apoptotic pathway and prolongs cell survival. Our study suggests that TP53 intronic SNPs might have strong influence on disease progression and poor response in CML patients. Hence, the study would be helpful in evaluating the progression and response status.

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