RESEARCH COMMUNICATION

TP53 Codon 72 Polymorphism and Risk of Acute Leukemia

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Abstract

***TP53* is the mostly commonly mutated gene in many cancers and the P53 tumor suppressor protein is involved in multiple cellular processes, including transcription, DNA repair, genomic stability, senescence, cell cycle control and apoptosis. A common single nucleotide polymorphism located within the proline rich region of *TP53* gene at codon 72 in exon 4 encodes either proline or arginine. *TP53* Arg 72 is more active than *TP53* Pro 72 in inducing apoptosis. The aim of this study was to understand the association of the 72 codon polymorphism with acute leukemia development and prognosis. A total of 288 acute leukemia cases comprising 147 acute lymphocytic leukemia (ALL) and 141 acute myeloid leukemia (AML), as well as 245 controls were recruited for analysis of the *TP53 72* polymorphism using PCR-RFLP method. Significant association of homozygous arginine genotype with AML was observed ($\chi 2$- 133.53; df-2, $p < 0.001$. When data were analyzed with respect to clinical variables, elevation in mean WBC, blast %, LDH levels and slight reduction in DFS in ALL cases with the arginine genotype was observed. In contrast, AML patients with Pro/Pro had elevated WBC, Blast%, LDH levels with slightly reduced DFS. Our study indicates that Arg/Arg genotype might confer increased risk to development of acute myeloid leukemia.**

Keywords: Acute leukemia - SNP analysis - *TP53* polymorphism - RFLP

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Introduction

The human *TP53* gene is located on short arm of chromosome 17p13, encompassing 20 kilo bases (Kb) of DNA comprising of 11 exons. It encodes for 1.53KDa nuclear phosphoprotein composed of 393 amino acids, highly conserved in diverse organisms. It is a tumor suppressor gene and frequently mutated in most of the cancers (Hollstein et al., 1996). The *TP53* protein is a transcription factor that regulates the expression of a wide variety of genes involved in cell cycle arrest and apoptosis in response to genotoxic or cellular stress (Donehower et al., 1993; Ko and Prives 1996). Growth arrest or cell death prevents damaged DNA from being replicated suggesting important role played by *TP53* in maintaining the integrity of the genome (Lane et al., 1992). This activity is central to its role as a tumor suppressor and also of major importance in the response of many cancers to conventional therapies which trigger apoptosis by damaging DNA. Inactivation of *TP53* through deletion, mutation or interaction with cellular or viral proteins is a key step in the development of over half of all human cancers (Nigro et al., 1987; Hollstein et al., 1996).

There is growing evidence that *TP53* also exerts its influence on multiple aspects of tumor development including suppression of metastasis and inhibition of angiogenesis. The *TP53* protein had been shown to limit angiogenesis by at least three mechanisms: Interfering with central regulators of hypoxia that mediate angiogenesis, inhibiting production of proangiogenic factors and directly increasing the production of endogenous angiogenesis inhibitors. The combination of these effects allows *TP53* to efficiently shut down the angiogenic potential of cancer cells. Inactivation of *TP53* which occur in approximately 50% of malignant tumors reverses these effects, as a consequence of which, tumors carrying *TP53* mutation appear more visualized, more aggressive and exhibit poor prognosis. Thus, the loss of functional TP53 during tumorogenesis likely to represent an essential step in the switch to an angiogenic phenotype that was displayed by aggressive tumors (Teodoro et al., 2007).

Several polymorphisms have been identified within *TP53* gene, both in non-coding and coding regions (Olivier et al., 2002). The Codon 72 of the *TP53* gene harbor a well known polymorphic site either with Arg or Pro in the hydrophobic midsection of the *TP53* protein. The two polymorphic forms (Pro72 and Arg72) of *TP53* gene have different primary structures and electrophoretic migration properties (Sreeja et al., 2008) with different biochemical and biological potentials including binding to transcriptional machinery and activation of transcription, but they do not differ in their property to bind DNA (Thomas et al., 1999; Dumont et al., 2003). This polymorphism had been shown to exhibit

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ethnic and geographical distribution (Weston et al., 1994; Beckman et al., 1994). It had been reported that the P53 Arg homozygous genotype could be a potential genetic risk factor for cancer. Initial studies had reported an association of codon 72 Arg/Arg and 7 fold increased risk of HPV associated cervical cancer. Invitro studies had indicated that HPVE6 oncoprotein targets readily the arginine form opposed to proline form of *TP53* for degradation (Crook et al., 1996).

The arginine (Arg 72) allele increases the ability of p53 to locate to mitochondria and induce cellular death, whereas proline allele (Pro 72) exhibits a lower apoptotic potential and an increased cellular arrest in G1 of the cell cycle (Bergamaschi et al., 2006).

Materials and Methods

The present study includes 288 acute leukemia cases comprising of 147 acute lymphocytic leukemia (ALL), 141 acute myeloid leukemia (AML) and 245 control samples. Blood samples were collected from acute leukemia patients being treated at NIMS (Nizams Institute of Medical Sciences), Hyderabad. The age and sex matched control samples were randomly selected from local population. Patient's clinical data like WBC count, blast%, platelet count, Hb, LDH, complete remission (CR) response to therapy and disease free survival (DFS) was noted from the tumor registry file with the help of medical oncologist. Genomic DNA was isolated by using salting-out method (Nuremberg and Lahari, 1991) and used for genotyping of *TP53 Codon 72* polymorphism through PCR-RFLP analysis.

Genotyping of TP53 Codon 72 polymorphism

TP53 codon 72 polymorphism was analyzed through PCR-RFLP (restriction fragment polymorphism length polymorphism) analysis technique. 131bp fragment was amplified using gene specific primer sequences: Forward: 5'-GAA GAC CCA GGT CCA GAT GAA-3', Reverse: 5'-GAA GGG ACA GAA GAT AGA CAG G-3'. The 25μl PCR reaction mixture consisted of approximately 100-150ng of genomic DNA, 15 pmol/l of each primer, 200μmol/l of dNTPs, 20 mmol/l of Tris HCl, 50 mM of KCl, 2.5 mmol/l of MgCl2, 0.5 U of Taq DNA polymerase and deionized water (varied). The PCR Cycling Conditions include initial denaturation at 940 C for 5 minutes followed by 30 cycles of denaturation at 940C for 1 minute, annealing at 600C for 2 minutes, extension at 720C for 3 minutes and final extension at 720C for 5 minutes. After amplification, PCR products were subjected to restriction digestion by 3U of Bsh1236I enzyme (Fermentas, India). The digested products were run on 3% agarose gel and bands were identified under UV transilluminator. The proline genotype produced a single 131bp fragment and arginine genotype produced two fragments of 81 and 30 bp and heterozygote produced all these three bands.

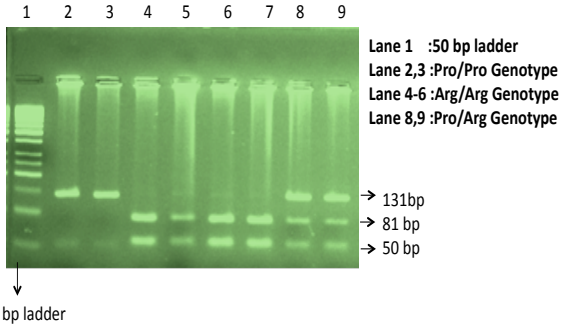

Figure 1. Gel Photograph of TP53 Codon 72 Polymorphism.

Table 1. Genotype Distribution of TP53 Codon 72 Polymorphism in Acute Leukemia and Controls

Genotype	Control (n=245)	ALL(147)[1]	AML (141)[2]
Frequency Genotype (n, %)			
PP	43 (17.6)	21 (14.3)	33 (23.4)
PA	123 (50.2)	67 (45.6)	44 (31.2)
AA	79 (32.2)	59 (40.1)	64 (45.4)
Allele			
P	0.43	0.37	0.39
A	0.57	0.63	0.61
Odds Ratios	ORs	(95%CI)	ORs (95%CI)
PP vs. PA	0.89	(0.49 to 1.62)	2.19 (1.226 to 3.924)*
PA vs. AA	0.73	(0.46 to 1.14)	0.45 (0.279 to .7115)*
PP vs. AA	0.66	(0.36 to 1.21)	0.95 (0.541 to 1.65)

'χ^2 value significant; *Odds ratios significant, [1]PA χ^2 =2.63, p-value=0.27, [2]PA χ^2 =13.19, p-value 0.001*

Statistical Analysis

All the statistical analyses were performed using Statistical Package for the Social Science (SPSS) 15.0. Chi square test was calculated to test the significance of genotype association with the occurrence of CML and its prognosis. All the P values were two sided and the level of significance was taken as P <0.05.

Results and Discussion

A common polymorphism in *TP53* gene, unique to humans is located within the proline rich region of *TP53* gene at codon 72 in exon 4 and encodes protein either with proline or arginine. The proline at residue 72 of *TP53* is part of PXXP motif that is known to be critical in containing the tyrosine residue of SH3 domain-containing proteins. *TP53* Arg 72 is more active than *TP53* Pro 72 at inducing apoptosis due to varied tendency of protein localization in mitochondria. The mutation frequency in *TP53* gene with Arg 72 allele is higher than the protein with Pro72. On the other hand, Martin et al., (2000) reported that the proline variant was more effective in inducing G1 arrest than the arginine variant, probably due to altered binding affinity to P53. A recent study demonstrated the influence of *TP53 codon72* polymorphism on DNA repair capacity indicating that *TP53* 72Pro variant activates several *TP53* dependent target genes involved in DNA repair more efficiently than the 72Arg variant expressing cells (Siddique et al., 2006). It has been suggested that the *TP53 codon 72* polymorphism may influence expression of the *TP53* gene since the substitution occurs within the *TP53* transactivation domain (Gottlieb et al., 1996; Wang et al., 1999). The codon 72 is located in the hydrophobic

Table 2. Mean Values of Clinical Variables with Respect

Clinical variables	P/P		P/A	
	Mean ±SE	n	Mean ±SE	n
TP53 Codon 72 Polymorphism in ALL group:				
Mean Age	7.29±2.78	21	16.64±1.29	67
Mean WBC (Thousand)				
	50.77±12.72	21	43.03±5.27	67
Mean blast%	45.71±6.98	21	47.96±3.98	67
Mean platelet count(lakhs)				
	0.82±0.16	21	0.89±0.08	67
Mean HB	8.71±0.64	21	8.91±0.29	67
Mean LDH	810.05±128.58	21	707.72±78.08	67
Mean DFS	31.67±4.72	21	26.50±2.69	58
TP53 Codon 72 Polymorphism in AML group:				
Mean Age	31.38±2.76	32	33.09±2.54	43
Mean WBC (Thousand)				
	*79.98±17.11	32	54.18±13.03	43
Mean blast%	69.75±4.12	32	61.14±4.06	43
Mean platelet count (lakhs)				
	1.08±0.24	32	1.01±0.20	43
Mean HB	8.60±0.45	32	7.97±0.42	43
Mean LDH	614.69±85.34	32	448.33±45.5	43
Mean DFS	10.27±2.51	15	11.69±1.54	26

* 'χ2 value significant; *F value significant ($p<0.05$).

region of the protein which determines its conformation, DNA-binding and transcriptional activity, essential for growth suppression (Greenblatt et al., 1994).

In the present study, it was observed that codon 72 polymorphism was significantly associated with AML cases compared to controls. The frequency of homozygous Arginine genotype was significantly elevated in the AML group compared to control group but no significant association was observed with ALL (χ^2-13.198; df-2, p -< 0.001*). This association might indicate that the hyper mutability reported for *TP53* gene with at codon72 Arg allele might lead to unstable protein and lack of tumor suppressor activity leading to myeloid malignancies. Previous studies found significantly increased risk for Arg 72 genotype to develop cancers such as cervical cancer (Crook et al., 1996), breast cancer (Beckman et al., 1994; Weston et al., 1994; Nurbuyru et al., 2003), adult T cell leukemia (Takeuchi et al., 2005). Further the TP53 Arg variant is less efficient in activating DNA repair genes compared to TP53 proline variant.

There was no association of sex of the patient with *TP53 codon 72* polymorphism. Hence the elevated frequency Arg/Arg genotype was also observed in AML patients with early age at onset <20 years.

When genotype data was analyzed with respect to clinical variables, significant elevation in LDH level was observed in ALL patients with homozygous Proline genotype, other variables showing insignificant trend. Whereas AML patients with Pro/Pro has elevated mean WBC blast%, LDH levels with slightly reduced DFS. There was no association between codon 72 polymorphism with the rate of complete remission failure in both ALL and AML.

In conclusion, significant association of Arg genotype with development of AML and Proline genotype with poor clinical progression was observed in the present study indicating that the Arg genotype with ability to induce mutation in P53 gene might trigger development of AML and Proline variant with less efficient apoptosis inducing ability responsible for progression of AML. However, ALL did not show significant association with *T53 codon72* polymorphism may be responsible for poor response to chemotherapy targeted to induce apoptosis leading to progression of AML.

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