

Mutational analysis of TP53 gene in Tunisian familial hematological malignancies and sporadic acute leukemia cases

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Abstract Mutations are responsible for familial cancer syndromes which account for approximately 5–10 % of all types of cancers. Familial cancers are often caused by genetic alterations occurring either in tumor suppressor or genomic stability genes such as *TP53*. In this study, we have analyzed the *TP53* gene by direct sequencing approach, in a panel of 18 Tunisian familial hematological malignancies cases including several forms of leukemia, lymphoma and myeloid syndrome and 22 cases of sporadic acute leukemia. In one familial case diagnosed with acute lymphoblastic leukemia, we reported an intronic substitution 559+1 G>A which may disrupt the splice site and impact the normal protein function. Most of the deleterious mutations (Arg158His; Pro282Trp; Thr312Ser) as classified by IARC data base, were commonly reported in ALL cases studied here. The cosegregation of the two variants rs1042522 and rs1642785 was observed in most patients which may be in favor of the presence of linkage disequilibrium. The most defined *TP53* mutations found here were identified in acute lymphoblastic leukemia context whereas only 3 % of mutations have been in previous

studies. The cosegregation of the two recurrent variant rs1042522 and rs1642785 should be further confirmed.

Keywords *TP53* gene mutations · Familial hematological malignancies · Acute lymphoblastic leukemia · Acute myeloid leukemia

Introduction

Since the discovery of *TP53* gene in 1979, several studies have defined this gene as a key tumor suppressor. This gene remains the most important factor in human cancer, by ensuring crucial cells process such as maintaining genomic stability, inducing cell-cycle arrest and assuring replicative senescence or apoptosis and DNA repair in response to various types of stress. The human p53 gene is located on chromosome 17p and consists of 11 exons and 10 introns [1, 2].

The *TP53* disruption was estimated to 50 % of all human cancers mostly in solid tumors [3, 4]. However lower frequency (10–20 %) was found in hematological malignancies according to IARC data base: 10–20 % of cases of chronic lymphocytic leukemia (CLL) [5], 10–12 % cases of multiple myeloma (MM) [6], 3–8 % of cases of acute myeloid leukemia (AML) [7] and less than 3 % in acute lymphoblastic leukemia (ALL) [8].

The *TP53* mutation was evoked in several familial cancer including breast-ovarian cancer, colorectal cancer and especially in Familial Li–Fraumeni syndrome in which 60–80 % carries *TP53* mutations. These mutations were often described in many studies targeting hematological malignancies, but not in familial aggregation context. According to the International Agency for Research in Cancer (IARC) *TP53* data base, several germline mutations

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were reported in several hematological malignancies showing highly polymorphic *TP53* gene in coding and non coding regions. Epidemiological studies carried out in hematological malignancies revealed a poor prognosis among patients carrying *TP53* mutation characterized by worse overall survival and resistance to chemotherapy [9] compared to those with wild-type form.

We hypothesize that patients with familial hematological malignancies might present *TP53* gene mutations. We aimed to determine to what extent the *TP53* gene is involved in familial context, compared to sporadic acute leukemia. The entire *TP53* gene was investigated in a panel of 18 Tunisian family cases with aggregated hematological malignancies by direct sequencing. 22 sporadic acute leukemias (13 ALL and 9 AML) were included in this study.

Patients and methods

The studied cohort consists of 18 patients belonging to 10 independent Tunisian families in which aggregation of hematological malignancy in first, second or third degree relatives were reported. The familial cases include 10 cases of Hodgkin lymphoma, 1 case of Chronic Myeloid Leukemia (CML), 2 cases of AML, 3 cases of ALL, 1 case of non hodgkin lymphoma (NHL) and 1 case of Myelodysplastic Syndrome (MS). The mutation analysis was performed from blood patients during complete remission according to the stander protocol of treatment of each pathology. Informed consent was obtained from the patients, relevant family members (healthy relatives) or their legal guardian as required by the Helsinki Declaration.

13 ALL and 9 AML sporadic Tunisian cases were included in this study. The mutational analysis was performed from blood patients during the diagnosis.

Genomic DNA was extracted from whole blood with the EZ1 DNA tissue kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The entire *TP53* gene was amplified using standard PCR methods. All primers and PCR conditions are available under request. The amplified PCR products were column-purified and both strands were sequenced using the BigDye Terminator Cycle Sequencing Ready Reaction Kit v1.1 (Applied Biosystems-Foster City, USA) and loaded onto an ABI Prism 3500 sequencer (Applied Biosystems). The sequence chromatograms obtained were compared to the published human *TP53* gene sequence (Genebank accession number NC_000017.10) using the SeqScape software program v2.5 (Applied Biosystems).

TP53 variants found were checked in *TP53* data base of IARC. The impacts of each variant were evaluated using bioinformatics tools provided in IARC database as SIFT,

Align AGVGD and Human Splicing Finder, PolyPhen-2 (Adzhubei et al. 2010) and MEGA-MD (Molecular Evolutionary Genetics Analysis software) <http://www.mega-software.net/mega-md/mega-md.php> [10].

Results

The *TP53* mutations were screened in 10 independent Tunisian families with aggregation of hematological malignancy and 22 sporadic acute leukemia cases. Among the 40 DNA analyzed, sequences alignment allowed us to identify 10 DNA variations: four mutations were intronic while the six other ones were exonic. Most of them was previously reported and listed in IARC data base. The results are summarized in Table 1.

We have identified four intronic variants in *TP53* gene: c.74+38 C>G; c.559+1 G>A; c.933+12 T>C; c.1100+30 A>T located respectively in intron: 2, 5, 9 and 10. The most recurrent intronic variant was the c.74+38 C>G found in 37/40 of cases including familial and sporadic AML or ALL cases in which 22 were homozygous. The c.559+1 G>A variant was found in only one familial case, diagnosed with B-ALL. This variant was classified as intronic mutation since it impacts the splice site of intron 5 of *TP53* gene.

Two synonymous variants of *TP53* gene were found in this study: Pro36Pro and Arg213Arg. The first substitution Pro36Pro was found in only one case of sporadic ALL, whereas the second one Arg213Arg was more recurrent and was detected in 9 patients including 6 familial cases among them 3 cases of hodgkin lymphoma (HL), 2 cases of ALL and one case of MS and 3 sporadic cases: 1 ALL and 2 AML in which one was homozygous.

We have detected four nonsynonymous substitutions: Pro72Arg, Arg158His, Pro282Trp and Thr312Ser. The Arg158His and Pro282Trp were found in two patients diagnosed with sporadic ALL. The Pro72Arg variant was the most recurrent substitution found in 36/40 analyzed cases, either in sporadic acute leukemia or familial hematological malignancies. Among the 36,19 patients were homozygous for Arginine amino acid. We notice that each patient carrying Pro72Arg substitution except one, carries also the intronic variant c.74+38 C>G, with the same degree of homozygosis in most cases. The Thr312Ser substitution (rs145151284) was found in only one case of sporadic ALL. The Sift analysis predicts a neutral effect on protein function.

Discussion

Among the driver Mutations described in several cancers, the *TP53* gene has the highest prevalence. Several somatic and germline inherited mutations were described referring

Table 1 Mutational analysis of TP53 gene in 40 analyzed cases of familial hematological malignancies and sporadic acute leukemia

Nucleotide change	Amino acid change	Exon/intron number	Occurrence	Context	Effect	Reference
c.74+38 C>G	–	2	37/40	FHM/AML/ALL	Intronic	rs1642785
c.215 C>G	p.Pro72Arg	4	36/40	FHM/AML/ALL	Missense	rs1042522
c.108 G>A	p. Pro36 Pro	4	1/40	ALL	Silent	rs1800370
c.473 G>A	p.Arg158His	5	1/40	ALL	Deleterious missense	rs139200646
c.559+1 G>A	–	5	1/40	FHM	Splicing mutation	–
c.639 A>G	p.Arg213Arg	6	9/40	FHM/AML/ALL	Silent	rs1800372
c.844 C>T	p.Pro282Trp	8	1/40	ALL	Deleterious missense	rs28934574
c.935 C>G	p.Thr312 Ser	9	1/40	ALL	Missense	rs145151284
c.933+12 T>C	–	9	2/40	ALL	Intronic	rs1800899
c.1100+30 A>T	–	10	1/40	FHM	Intronic	rs17880847

AML, ALL: sporadic leukemia cases

FHM familial hematological malignancies

to IARC TP53 Data Base and literature. A large study screening 525 patients with several cancer family history, has identified 17.3 % of patients carrying *TP53* germline mutations [11].

According to IARC data base, 3.37 % of hematological malignancies were associated with germline *TP53* mutations. This data insight us to establish the mutational screening of *TP53* gene in familial hematological malignancies since to our knowledge no study was carried out in familial context. For this we sequenced the entire *TP53* gene in 22 Tunisian sporadic leukemia cases including AML and ALL and 18 Tunisian familial hematological cases including 10 cases of HL, 1 case of CML, 2 cases of AML, 3 cases of ALL, 1 case of NHL and 1 case of MS.

We report here 10 *TP53* variants, 4 of them are located in introns and 6 in exons. According to IARC data base, 90 % of known polymorphisms are identified in *TP53* introns including all type of cancer. In our investigation conducted on hematological malignancies context, we found more variations in exons than in introns.

Intronic variants

Among the 4 intronic variants, one interesting mutation was found: the c.559 + 1 G>A located in the fifth intron. It might interrupt the RNA splicing process. For this reason, it was classified as *TP53* splicing mutation [12]. The c.559 + 1 G>A mutation was found in one single familial case diagnosed with B-ALL at the age of 39. This patient showed a worse outcome due to bone marrow infiltration and chemical treatment resistance and died. His 20-years old cousin (first degree relative) was also diagnosed with ALL and also showed a worst outcome with drug resistance. After bone marrow transplantation failed, she relapsed and died. She was not included in this study since

the DNA was not available. This mutation may be associated with poor prognoses.

We have found the c.74+38 C>G (rs1642785) variant localized in intron 2 in 92 % of cases including familial cases and sporadic AML/ALL. A recent new role was attributed to rs1642785 on mRNA splicing and stability, and thus on the differential expression of isoform-specific transcripts of the *TP53* gene [13]. In only one study, this variant was associated with the Pro72Arg (rs1042522) one to glioma in indian population [14].

The c.933+12 T>C (rs1800899) variant located in the ninth intron was found in two patients diagnosed with sporadic ALL. It was not assigned in IARC database. It was previously described in patients with early onset of Colorectal Cancer [15] and in three breast cancer patients [16]. No significance of this change was described.

In one familial case diagnosed with T-ALL, we have found other intronic variant: the c.1100+30 A>T (rs17880847) which was already described in one study in one patient with malignant salivary gland neoplasms [17].

Exonic variants

We have identified in 40 cases of hematological malignancies analyzed here 6 exonic variants: 2 were Synonymous and 4 non synonymous mutations. The first synonymous substitution was c.108 G>A p.Pro36Pro (rs1800370), located in exon 1, was found in one single case of ALL. According to IARC data base, this variant was classified as a silent mutation. It was detected in 2.4 % of analyzed cases of glioblastoma but was totally absent in Indian controls population [14]. It was previously shown that the rs1800370 silent mutation reduces the ability of *TP53* to activate apoptosis by decreasing the affinity of *TP53* mRNA for MDM2, consequently reducing *TP53* levels [18].

The second synonymous mutation c.639 A>G p.Arg213Arg (rs1800372) located in exon 6, was found in 9 patients: 1 case of sporadic ALL and 2 cases of sporadic AML in which one was found at homozygous level, and 6 familial cases with several hematological malignancies. This variant was classified as silent polymorphism according to IARC data base with medium frequency.

Four missense substitutions were found in our investigation. The first one was c.473 G>A Arg158His (rs139200646) located in exon 5 described in one sporadic ALL. This variant was classified as deleterious missense substitution. The SIFT analysis is in favor of deleterious effect. Several studies have reported this variant in glioblastoma cases and Li–Fraumeni syndrome [19].

The second variant was c.844 C>T, p.Pro282Trp (rs28934574), located in exon 8 and found in one sporadic ALL. This deleterious mutation as classified in IARC data base is located in the DNA binding *TP53* core domain. Functional study have showed that it may reduce the DNA binding ability and leads to the loss of transactivation activity of *TP53* gene [20].

The third variant found also in sporadic ALL was c.935 C>Gp.Thr312Ser (rs145151284) located in exon 9. According to the IARC data base this variant is classified as missense substitution with neutral effect according to SIFT analysis.

The fourth variant c.215 C>G p.Pro72Arg (rs1042522) located in exon 4 was the most frequent and found in 90 % of cases including sporadic and familial cases. 19 of them were homozygous Arg/Arg. *TP53* rs1042522 is one of the most commonly investigated variant in cancer genetic epidemiology [21]. Several association studies have been conducted with many controversies about the pathogenicity effect of this variant. Meta-analysis have suggested that this polymorphism might be a risk factor for breast, colorectal, ovarian, or endometrial cancer and that the Pro allele might cause a small increase in the risk of lung cancer [22]. Among analysis targeting this variant in Tunisian population, an association study of rs1042522 variant conducted by Mabrouk et al. [23] has found that there is no significant difference in frequency distribution of genotyping alleles between 2 groups of Tunisian patients with bladder cancer and breast cancer in comparison to a control population. In a larger group of Tunisian breast cancer, a recent association study showed that *TP53* codon 72 polymorphism is involved in susceptibility to develop breast cancer. However, there is no evidence indicating that Arg72Pro SNP may influence response to anthracycline-based chemotherapy [24].

We report in this study that all patients except one, carrying the Pro72Arg variant present also the intronic variant c.74+38 C>G (rs1642785). Most of them were homozygous for these 2 variants, which may suppose a linkage disequilibrium which has to be furtherer confirmed.

TP53 mutations were not much investigated in ALL compared to other cancers, but worst outcome were reported in patients carrying *TP53* mutation. This poor outcome was observed in all patients diagnosed with ALL and carrying *TP53* mutation [25].

Despite the low frequency 3 % of *TP53* mutation reported by previous epidemiological data in sporadic ALL [8], in our investigation most of *TP53* mutations were found commonly in patient diagnosed with sporadic ALL compared to sporadic AML and Familial hematological malignancies. Further *TP53* investigations targeting sporadic Tunisian ALL are needed.

Conclusion

Germline mutations are responsible for familial cancer syndromes which account for approximately 5–10 % of all types of cancers. These mutations mainly occur within tumor suppressor genes as *TP53*. Somatic *TP53* mutations were included in almost every type of cancer whereas germline mutations were described in familial cancer context like Li–Fraumeni syndrome. The *TP53* gene was screened in several hematological malignancies but not in familial aggregation context. In this study we have target for the first time the *TP53* gene in 10 independent families. We reported only one intronic deleterious mutation c.559+1 G>A. The most defined *TP53* mutations found here were identified in sporadic ALL whereas only 3 % of mutations have been reported in literature. This finding will encourage us to investigate the *TP53* gene in a larger Tunisian ALL cases. We aimed also to focus on the cosegregation of the two most frequent variants rs1042522 and rs1642785 through a linkage study.

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Authors' contributions W.S.H., S.B., and V.B. conceived the study and performed the experiments and wrote the manuscript, Y.B.Y. and M.A.L. provides simple and clinical data, T.N. analyzed the data, A. K., H.S. and Z. S. supervised the project.

Compliance with ethical standards

Conflict of interest We declare no conflict of interest.

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