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Determination of Myc Genes Mutations in Patients with Leukemia from Turkey

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Research Article

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ABSTRACT

Myc oncogenes are contributing to the development of cancer, and many of the genes to become active. Studies on the expression of these genes have led to the development of new therapies for the treatment of cancer patients. This situation was quickly discovered that A pioneer or biomarker Myc expression triggers the differentiation and proliferation of cells as mitogen-independent, especially. Myc activation was a consequence of mutations in the coding sequence. Mutations in the coding sequence were observed in 4 of the 50 leukemia specimens. Four mutations which is named SNP (rs3134614, rs1800834, rs61731506, rs74067837), in exon 2 mutations. DNA sequencing reveals that A, G, C, and T formed in exon 2 of the L-myc gene. Any change has been determined at exon 1. A point mutation or single point mutations called SNP contains an incorrect base pairing. In addition to, seeing if the point mutations of L-Myc have acute leukemias, or acute leukemia patients with the existence point mutations of L-Myc gene we analyzed by the gene sequence assay and Polymerase Chain Reaction-based Single-Strand Conformation Polymorphism (PCR-SSCP) assay. In the study, the mutation observed in patients with leukemia has proven to be the most common type of L-Myc mutations, consistent with the literature. There is significant difference in the genotype distribution between leukemia patients and controls was observed. Our results suggested that L-Myc gene polymorphism was a suitable prognostic marker in the identification of metastatic growth chronic lymphocytic leukemia (CLL) and acute myeloblastic leukemia (AML) in Turk populations.

INTRODUCTION

Leukemia is a malignant disease (cancer) of the bone marrow and blood. According to the type of leukemia cells are divided into four categories. These are acute or chronic myelogenous and acute or chronic lymphocytic leukemia. Growth of a cell that is associated with the proliferation as well as controlled short survival or proliferation of abnormal/ uncontrolled cell DNA. Accordingly, there are malignant or normal cells or cell shape. It determines the fate of cell any mutation in the DNA of cells [1-7]. In cases of leukemia observed uncontrolled increase in the number of lymphocytic cells in bone marrow leads to an increase in the number of lymphocytes in the blood at the same time. The leukemic cells that accumulate in the marrow in chronic lymphocytic leukemia do not prevent normal blood cell production as profoundly as in the case of acute lymphocytic leukemia. This important distinction from acute leukemia accounts for the less severe early course of the disease. The myc proto-oncogenes regulating the cell proliferation and differentiation dependent DNA origin phosphoprotein that is encoded by the core parts. Myc gene naturally mainly plays an active role in hematopoietic diseases such as leukemia and lymphoma. Studies in recent years, the myc gene family from the N-myc and L-myc gene that can cause normal and malignant hematopoiesis has also proved [7-11]. L-Myc gene first was discovered with structural similarity with C-Myc and N-Myc in small cell lung cell line. L-Myc gene was initially identified as a gene with structural smilarity to c-myc and N-Myc from a human small cell lung cancer line [12-16]. L-Myc is an oncogene localized to chromosome 1p34 and it passes activated during tumor growth [17-23]. L-Myc encoded by the gene and acts as a transcription factor these specific molecule or protein to which a nuclear phosphoprotein regulating the cell proliferation and differentiation.

In addition to early detection of cancer in individuals and transplacental treatments for identifying susceptibility to cancer gene mutations or polymorphisms determining their Imyc it is important. Therefore Imyc oncogene in Turk populations in this study has investigated whether a biomarker. Studies on cells showed that the protein from the N-terminus of myc resulted in point

mutations resulting in the uncontrolled proliferation of cells and thus inhibiting the cellular apoptosis showed that tumor growth was observed. Therefore, leukemia cancer has been attributed to many genetic abnormalities, including mutation/deletion of tumor suppressor genes, the existence of multiple autocrine growth cycles, and the proliferation and transcriptional deregulation of the cell. SSCP has a very high power to detect mutations in the single-chain DNA fragments the electrophoresis technique. SSCP analysis of one (or more) based on the ability to detect mutations or polymorphisms of the DNA are a commonly used technique. Single stranded DNA molecule which is not under denaturing conditions, the electrophoretic mobility is defined in accordance with changing circumstances [20]. In SSCP analysis in this study, PCR products radioisotope has been used to label non-fluorescent dye which are then the results are then interpreted by reading capillary electrophoresis. In this case, allows the formation of different peak or band profile of the heterozygous state. For most of the gene loci this Tris-formiate/borate buffer system gives sufficient discrimination power, to detect most of the mutations. The important point, the temperature of the reaction should be optimized for each of gene locus.

The objective of this study is to define the role of L-Myc mutations in leukemia pathogenesis of the Turkish population and also that is to emphasize and could be used as a biomarker of these genes. In the present study, we have analyzed the L-Myc mutations in 50 patients with leukemia possibility of Turkey. We therefore anayzed 4 patients for the single stranded DNA in order to identify the presence of mutations that conformational polymorphism or SSCP followed the mutations that activate the intercellular signaling pathway genes, including PTHC, identify DNA sequence analysis was performed. We measured the frequency of L-Myc polymorphisms alone, and in combination, and identified 4 novel base changes. To illustrate the applicability of these techniques to the screening of DNA mutations or polymorphisms, Later, SSCP to identify the best L-Myc mutation was also subjected to sequencing reaction have been tested sequentially. Briefly, in this study all methods were studied three times.

MATERIALS AND METHODS

Patients

Peripheral blood samples were collected from 50 patients with leukemia. The diagnosis of acute leukemia was based on morphological and cytochemical criteria of the Selcuk University, Faculty of Medicine, Department of Hematology, Konya, Turkey. Detailed medical history, physical examination and pathological diagnosis were performed for all patients in the study. A total of 50 patients and 50 healthy blood samples primary were obtained from the Department of Pathology and Hematology University of Selcuk, Medical Center, Turkey.

Genomic DNA Isolation

Genomic DNA was extracted from the peripheral blood of 50 patients with leukemia and 50 normal volunteer controls by using automatically EZ1 automatic DNA isolation system (QIAGEN GmbH, Hilden, Germany) with Qiagen DNA isolation kit. After extraction, absorbans ratio of DNA (A260/A280) was recorded by Shimadzu UV-1700 spectrophotometer to contol amount and purity of extracted of DNA from blood **(Figure 1)**.

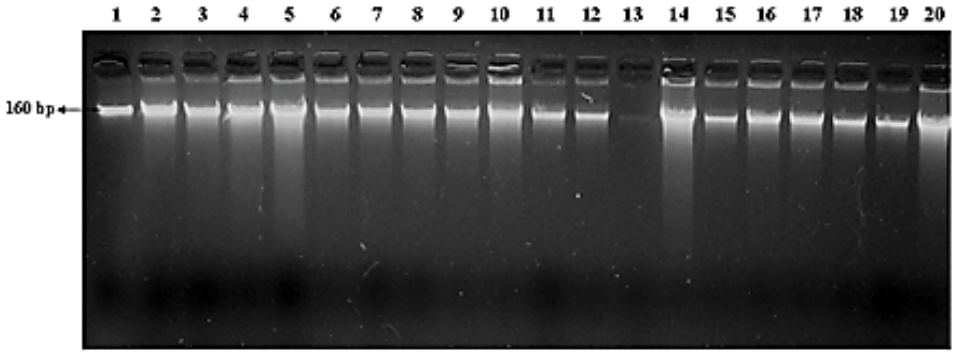

Figure 1. Genomic DNA was isolaled from patients with leukemia and for control healthy blood specimens, respectively, Lane 1, 2, 3-20 with BioRobot EZ1. DNA samples submitted to electrophoresis in 1% agarose gel.

Polymerase Chain Reaction (PCR) Amplification on L-Myc Exon1

Template DNA (0.5-1.0 µg) was used in a PCR under sterile conditions. 100 ng of primer was used for the reaction. PCR was performed using 100 ng of genomic DNA extracted from peripheral leukocytes and specific primer pairs:

Forward primer, 5'-AGTTCACTCACAGGCCACAT-3' and Reverse primer, 5'-TGCATATCAGGAAGCTTGAG-3' were used as primers (1 mM each) in a 25 µl PCR mix containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 50 mM $MgCl_2$, dNTPs (dGTP, dATP, dTTP, dCTP) at 0,5 mM each and 1 U of Taq polymerase. Amplifications were performed using an automatic thermocycler (BioRad) with initial denaturation steps at 94°C 60 seconds, followed by 30 cycles (94°C for 30 sec, 50°C for 1 min, and 74°C for 1 min). The

PCR products were separated by gel electrophoresis in a 2% agarose solution containing ethidium bromide and were imaged on UV transsimilator. Afterwards, The amplified 267 bp PCR products were directly digested with the restriction enzyme EcoRI (Fermentas).

Genetic Markers

SNPs were identified in a pilot study of 50 selected Turkey Leukemia patients using PCR amplification and comparison of nucleotide sequences. PCR primers were designed to amplify 267 bp DNA fragments covering the entire coding region and 2.8 kb of the noncoding sequence of L-Myc (Genbank accession M19720). DNA samples were amplified in a final reaction volume of 25 ml containing 50 ng genomic DNA, 100 mM dNTPs, 1.5 mM $MgCl_2$, 1 U AmpliTaq Gold Polymerase (Fermentase) and 10 pmol of each specific primer. Thirty-five PCR cycles, each consisting of 15 sec at 95°C, 10 sec at 58°C and 20 sec at 72°C, were carried out in the GeneAmp PCR System 9700. Aliquots of PCR products were loaded on ethidium bromide stained agarose gels, to check the quality of PCR amplifications **(Figure 2)**. Nucleotides were sequenced using an ABI PRISM 310 automatic sequencer (Perkin-Elmer), aligned and compared to identify SNPs.

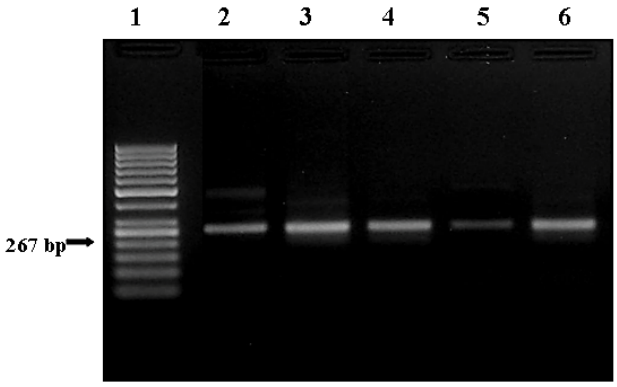

Figure 2. Direct visualization of PCR products by ethidium bromide staining. A 267 bp L-Myc fragment was amplified, and electrophoresed on a 2% agarose gel. Lane 1, 50 bp ladder size standard. Lane 2-5, PCR products of leukemia patients, and Lane 6, PCR products of healthy person.

Mutational Analysis of the L-Myc Oncogene by SSCP

DNA samples of patients with leukemia were amplified for the SSCP analysis of the L-Myc gene using PCR. Mutation analysis of L-Myc was performed for exons1 to five samples using polymerase chain reaction (PCR) primers binding to the intronic sequences flanking each exon. The PCR products of each exon were screened for mutations by single strand conformational polymorphism (SSCP) or heteroduplex analysis. After amplification, 10 ml of PCR products were mixed with 20 ml loading buffer (95% formamide, 20 mM EDTA, 0.05% xylene cyanol and bromophenol blue), denatured at 95°C for 10 min, and quenched on ice. For the SSCP analysis, after heat denaturation the PCR products were subjected to electrophoresis on nondenaturating polyacrylamide gels. The SSCP or heteroduplex band patterns of patients and normal blood were visualized by silver staining of the gels **(Figure 3)**.

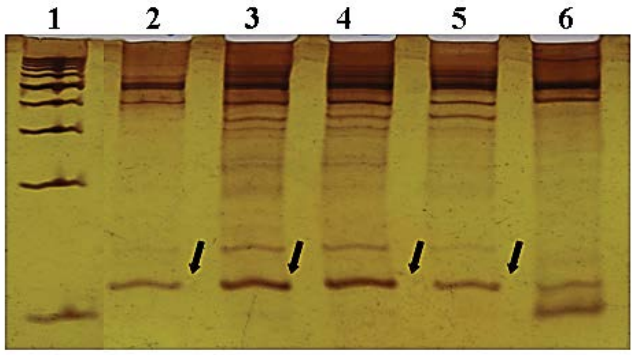

Figure 3. Single strand conformation polymorphism based-polymerase chain reaction (SSCP) analysis of L-Myc gene, respectively, lane 1, 50 bp ladder size standard, and lane 2-5, mobility shifts due to mutations in PCR products of leukemia patients, lane 6, PCR products of healthy person using for control.

DNA Sequences

In this study, once defined in SSCP heteroduplex analysis of PCR products pure examples of automatic DNA sequence analysis apparatus with which analysis was performed using ABI PRISM 310 DNA sequencing directly. Purified PCR products of the samples showing mobility shift on SSCP analysis and randomly chosen samples were used for direct DNA sequencing using

Automated DNA sequencer ABI PRISM 310. PCR using primers forward and reverse sequence occurs to reduce the artifact was made at least two times PCR was performed and optimized PCR products are ready to react. And then the Applied Biosystems based on fluorescence labeling was performed by sequence analysis. Purified single-stranded extension products were then resolved on ABI PRISM 310 or DNA Sequencer **(Figures 4 and 5)**.

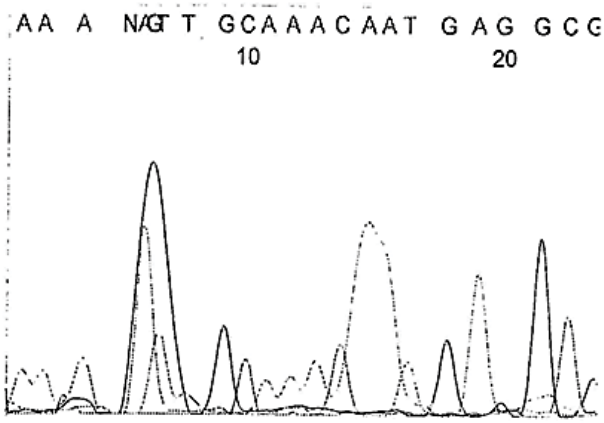

Figure 4. Sequence electrogram of leukemia cancer patients showing mutation heterozygote sequence for L-Myc gene in exon 2.

aaanagttgc aaacaatgag gcgggggggtg gtgaaagtga tctggcagca gagctcaccc

aataggggct aggggctggg taagacagaa gtccaaacac agcgtaatca gccaatcatg

ggctttgggg ccaggagggc tgaatggtca ggtttattaa tggagaaata atgcgattgt

ccacacaatg gaagccttcc tgacaaaggg gctcaagctt cctgatatgc aatc

Figure 5

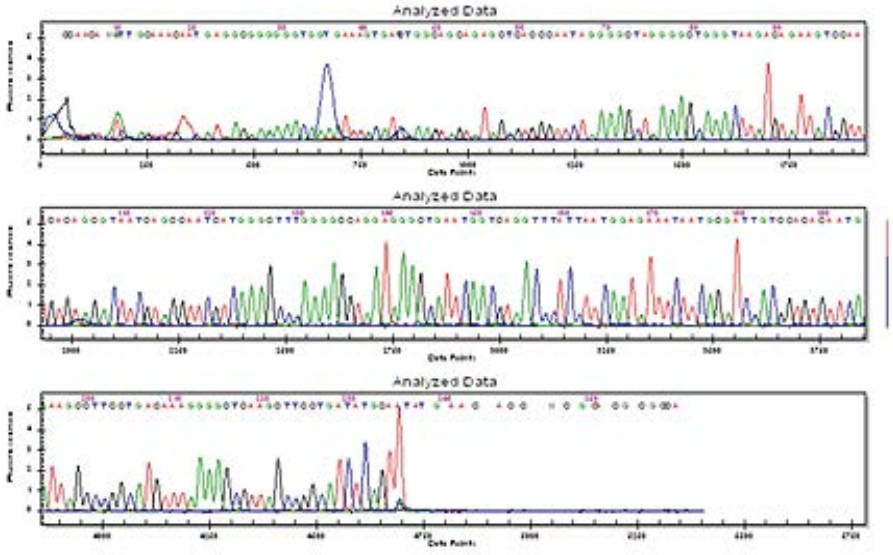

Figure 5.1

Figure 5. General screening of mutation loci in L-Myc gene sequence in patients with leukemia from Turkey population.

Statistical Analysis

According to the results of statistical analysis, the social sciences 10.0 software package (SPSS) using version. Then gene changes between patient and exacting Fisher test was used for non-disease individuals who were not found were evaluated and compared with the clinical and pathological parameters. The association between the development of clinical metastases/ recurrence and the presence of serum molecular markers was also evaluated by the two-sided Pearson 2 test. A probability of less than 0.05 was considered to be statistically significant.

RESULTS AND DISCUSSION

Most types of cancer cellular mutations are found in DNA and significant genomic variations occurring in the defined region of said gene consists result and their condition was modified molecular and biochemical assays. Mutations that play an important role in the development of cancer cells is very diverse. Especially inversion in addition to abnormal cell proliferation in leukemia and most common chromosomal translocations or abnormalities and these mutations, suggesting the formation of significant leukemic clone. Human cancers, some oncogenes show various abnormalities such as point mutation [8] amplification [10], rearrangement [10,12], and overexpression [9,12]. Among them, abnormalities of the Myc gene family (c-myc, N-myc, and L-myc) gene amplification, have frequently been detected in various cancer tissues and cell lines such as leukemia [6,8,18], and cancers of the colon [13], stomach [15,21], breast [14,16], and lung [17]. The c-myc from myc gene family plays an important role in the control of cell proliferation, differentiation, and apoptosis, and myc gene expression observed in the abnormal situation is very common in human cancer [19,20,24,25]. The polymorphic L-Myc gene locus was analyzed by PCR-RFLP in 50 patients and 50 healthy individuals. The three genotypes were the LL homozygote appearing as a 267 bp, the LS heterozygote with 267, 140 and 120 bp and the SS homozygote with 140 and 120 bp fragments. In this study, we have identified L-Myc mutation and no relationship between stages of disease, also in the article, respectively; we analyzed 100 individuals recruited among healthy blood donors and patients. One aim of the present study was to investigate the frequency to identify polymorphisms in the L-myc in the Turkish people and also a mutation identification technique to standardize the SSCP technique. There was no statistically significant difference between various types of leukemia regarding to L-Myc gene polymorphism. Mutations were detected in 4 of the 50 leukemia specimens. Four mutations which is named SNP or rs3134614, rs1800834, rs61731506, rs74067837, in exon 2 mutations. DNA sequencing reveals that A, G, C, and T occurred in exon 2 of the L-Myc gene. Any change has been determined at exon 1. Namely, Exon 1 did not show any mutation, regardless of the presence or absence of loss of heterozygosity. In addition to, we used the same time as the molecular genetic techniques to determine whether point mutations in L-Myc gene are involved acute leukemias the PCR-based Restriction Fragment Length Polymorphism (PCR-RFLP) techniques. Later, we analyzed by the gene sequence assay and Polymerase Chain Reaction-based Single-Strand Conformation Polymorphism (PCR-SSCP) assay. Single nucleotide polymorphism (SNP) markers being the most common polymorphism observed in a genome. Another objective of this study were to discover SNPs in gene sequence and determination of L-myc gene mutation and polymorphism in patients with leukemia by comparing sequences from coding and noncoding regions obtained from the GenBank and genomic DNA and to compare sequencing results with those obtained using single base extension assays. Single nucleotide polymorphisms in coding regions may have functional significance if the resulting amino acid change causes altered phenotype. All leukemias and their normal healthy specimens were screened for the mutations of the L-Myc oncogene in two exons using PCR-SSCP analysis and of those patients; four cases were detected in exon 2 and SNP sites (rs3134614, rs1800834, rs61731506, rs74067837) in exon 2 mutations. DNA sequencing reveals that A, G, C, and T occurred in exon 2 of the L-myc gene **(Figures 4 and 5)**. In this study, we analyzed 100 individuals recruited among healthy blood donors and patients. However, we found a statistically significant difference in allele frequencies among patients with benign and malign situation. There are no previous studies must be it is known that mutations of genes involved in the control of cellular growth and/or differentation such as c-myc affect the development of leukemia [3,5]. The frequency of patients was significiantly higher in the study population, as well as in the groups of other cancer, which is in agreement with the gender distribution usually observed in this type of cancers [11]. The SSCP technique has been shown to be useful and economical for the detection of a series of mutations and polymorphisms in the gene [4,17,18], Despite the use of the technique it is limited. Detection of single base substitutions is less sensitive in larger DNA fragments [24], and it may be advantageous to select other primer pairs or digest larger fragments to generate DNA molecules of the optimal size defined Collins et al [4] as 150 bp. In conclusion SSCP has been successfully used to locate sequence variants in L-Myc. The finding of a number of different mutations excludes the use of mutation analysis for prenatal diagnosis, but further investigation of the polymorphisms may yield useful markers for this purpose. A recent study showed that the point mutations inactivated the apoptosis activity of MYC and contributed to the tumor transformation. To see whether the point mutations of MYC are involved in cancer, we analyzed the N-terminal cluster region of the mutations in 50 cases with acute leukemias, by polymerase chain reaction-based single-strand conformation polymorphism assay. Summarize is to say, there was no somatic mutations in MYC gene in different cancers.

The data suggest that the point mutation of the MYC gene in the N-terminal domain may be very rare in common human cancers, although observed the presence of the point mutation in MYC gene was identified that contribute to cancer d.

CONCLUSION

We studied 50 leukemia patients and 50 healthy controls of Turkish origin for L-Myc gene polymorphism. For this reason, we studied determining the distribution of L-Myc gene polymorphism in Turkish patients with leukemia cancers and optimization of DNA isolation from patient and healthy blood samples and amplification of L-Myc gene loci using PCR. Furthermore, it was observed mutation in exon 2 of gene and whether is important L-Myc marker in leukemia patients, we analyzed 50 leukemia specimens. Mutations were detected in 4 of the 50 leukemia specimens. Four mutations which is named SNP (rs3134614, rs1800834, rs61731506, rs74067837), in exon 2 mutations. DNA sequencing reveals that A, G, C, and T occurred in exon 2 of the L-myc gene. Any change has been determined at exon 1. Namely, Exon 1 did not show any mutation, regardless of the presence or absence of loss of heterozygosity. A point mutation or SNP (Single Nucleotide Polymorphism also an incorrect base pairing occurs during the replication process. In addition to, we used a molecular genotyping method to see whether the point mutations of L-Myc are involved acute leukemias, we analyzed by the gene sequence assay and Polymerase Chain Reaction-based Single-Strand Conformation Polymorphism (PCR-SSCP) assay. The frequency of mutations was higher in the regions of the gene and missense mutations was regarded as the most common type of mutations causing severe leukemia and this conclusion was in accordance with the literature. Our results suggested that L-Myc gene polymorphism was a suitable prognostic marker of metastatic development in Turkish chronic lymphocytic leukemia (CLL) and acute myeloblastic leukemia (AML).

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