

## TP53 hotspot mutations in astrocytoma

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### ABSTRACT

The aim of this project was to investigate the P53 gene mutations in exon 8 in the patients with astrocytoma type brain tumor. This was an *in vitro* study, in which the extraction of DNA from 30 samples of brain tissue was performed by phenol-chloroform protocols. After polymerase chain reaction (PCR) amplification of exon 8 of p53 genes, screening by single strand conformation polymorphism (SSCP) was performed to detect the shift. In this study, three malignant novel missense mutations in one sample with grade 4 of astrocytoma were detected. Also one synonymous mutation in grade 4 of astrocytoma was detected. The findings were indicated that mutations in exon 8 of the P53 gene mostly were in malignant astrocytoma brain tumor type, which is important in diagnosis and gene therapy of brain tumors.

**Keywords:** Astrocytoma, p53, exon 8, mutation

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### INTRODUCTION

Astrocytomas are known as glioma and could be found in most parts of the brain and

especially in the spinal cord. Astrocytomas in parts of the brain are current in young people and estimated 75 % of neuroepithelial

tumors. The world health organization (WHO) system established a four-tiered histologic grading guideline for astrocytomas that assigns a grade from 1 to 4, which 1 was the least aggressive and 4 was the most aggressive [1].

Meningioma is regular intracranial tumor arising from the meningotheelial cells. Meningiomas could be found in 15 histologic subtypes and three grades, including benign (grade I), atypical (grade II), and anaplastic (grade III) [7]. Most meningiomas are benign [5-8].

P53 is a key regulator of stem cell homeostasis that impacts an array of cellular functions including genomic surveillance, cell-cycle regulation and apoptotic and inflammatory response. TP53 mutations are well described in human malignancies with up to 50 % of all cancers harboring a mutation in the DNA-binding domain or in one of its regulators affecting P53 function [2, 3].

Moreover immunohistochemical analysis of 82 neuroblastoma tumors showed an association of high P53 expression with MYCN expression and amplification. We concluded that MYCN transcriptionally upregulates P53 in neuroblastoma and uses P53 to mediate a key mechanism of apoptosis [9].

In a study, which was conducted on 85 samples of human glioma demonstrated that the P53 gene was mutated in 19 out of 85 (22 %) samples as determined by single strand conformation polymorphism (SSCP) analysis and DNA sequencing. It was also noted that hyper-methylation of P53 result in inhibition of apoptosis [11].

Since the P53 suppressing tumor gene has a role in protecting the genome and the MYC transcription factor that plays a critical role in cell cycle. On the other hand the few studies were done in this area therefore, in this study evaluated of exon 8, P53 mutation as tumor suppressor gene in patients affected with astrocytoma.

## **MATERIALS AND METHODS**

This applied study was performed on 30 Iranian astrocytoma brain tumors patient have been referred from the department of Neurosurgery at Shariati hospital in Tehran University of medical sciences.

### **DNA extraction**

Genomic DNA of human brain tumors samples isolates was extracted using phenol-chloroform method [1]. The concentration DNA was measured at 260 nm using nanodrop (Thermo scientific, USA).

### PCR primers and PCR amplification

Two PCR primers (5'-CCCTTGGCAACCAGCCCTGT-3' and 5'-ACAGGGCTGGTTGCCAAGGGG-3') were designed. The primers were synthesized by integrated DNA technologies (Coralville, USA).

PCR amplification was undertaken using the purified genomic DNA on one punch of the FTA paper, 0.25  $\mu$ M of each primer, 150  $\mu$ M of each dNTP (Bioline, London, UK), 2.5 mM of  $Mg^{2+}$ , 0.5 U of DNA polymerase (Qiagen, Hilden, Germany) and reaction buffer supplied in a 20- $\mu$ L reaction. The thermal profile for amplification consisted of 5 min at 94 °C, followed by 30 cycles of 30 s at 94 °C, 30 s at 56 °C and 30 s at 72 °C, with a final extension of 5 min at 72 °C. This was done in S1000 thermal cyclers (Bio-Rad, Hercules, CA, USA).

Amplicons were visualized by electrophoresis in 2 % agarose gels, using buffer and stained with ethidium bromide.

### Variant screening and sequencing

PCR amplicons were subject to SSCP analysis. A 5  $\mu$ L of each amplicon was mixed with 5  $\mu$ L of loading dye (98 % formamide, 10 mM EDTA, 0.025 % bromophenol blue, 0.025 % xylene-cyanol) and after denaturation at 95 °C for 5 min, the samples

were cooled on ice and loaded on 16 cm  $\times$  18 cm, 10 % acrylamide:bisacrylamide gels. Electrophoresis was performed at 200 V for 18 h at 25 °C in 0.5 buffer.

### Sequence analysis

DNA sequence analysis were carried out using Chromas software and a BLAST search was taken of the NCBI GenBank databases using the sequences identified, to find homologous sequences.

### Flowcytometry

Cells were rinsed twice with PBS, and centrifugation for 5 min, and then resuspended in 500  $\mu$ L of PBS. Approximately  $5 \times 10^5$  cells per 100  $\mu$ L were labeled with primary antibodies against P53 at 4 °C for 30 min and washed. The labeled cells were analyzed using a flow cytometer (Beckton Dickinson, San Jose, CA, USA). The antibodies used in this experiment were: P53-FITC (Beckton Dickinson, San Jose, CA, USA). All samples were run on a BD LSR II flow cytometer and analyzed using FlowJo Software.

## RESULTS

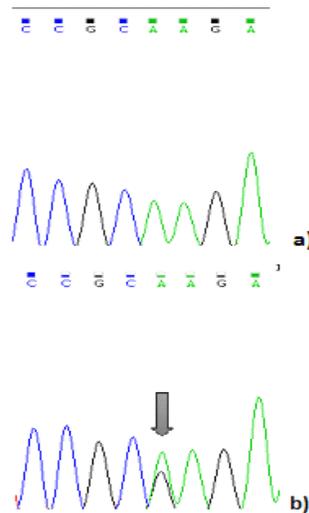
In this study, 30 samples of astrocytoma brain tumor were analyzed. After screening by SSCP technique (Figure 1), screened samples were sequenced. Results showed nucleotide changes A to G (g.13851A> G)

(Figure 2), C to G g.1386C>G and G to T g.13835G>T, observed at heterozygous state in one astrocytoma (grade 4) sample. A synonymous variation (g.13832A>G) was

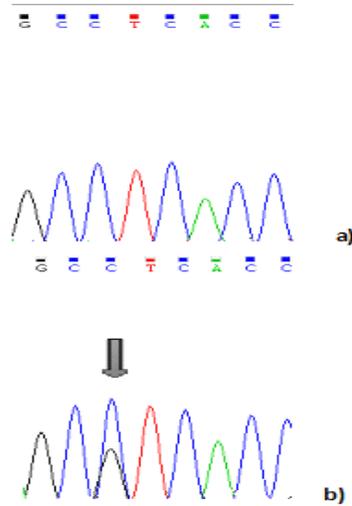
detected in grade 4 astrocytoma sample. These nucleotide changes have been confirmed by sequencing using reverse primers.



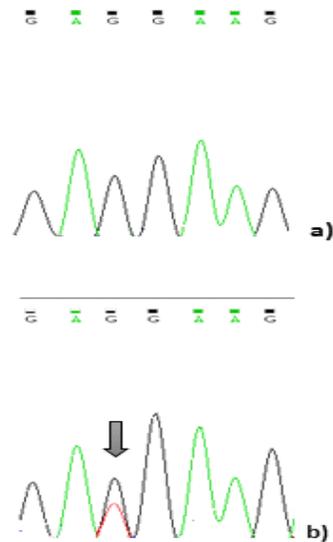
**Figure 1.** Gel electrophoresis of PCR-SSCP products of the p53 gene. (M=Marker (ladder 50bp), N=Normal, ND=non denaturated, for sequencing= 17, 26).



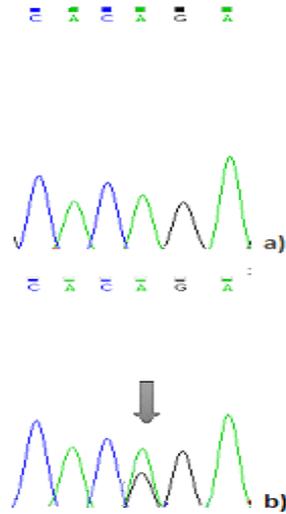
**Figure 2.** Sequencing results g.13851A> G (possibly damaging) missense mutation observed in one astrocytoma (grade 4) patient compared to normal sequence.



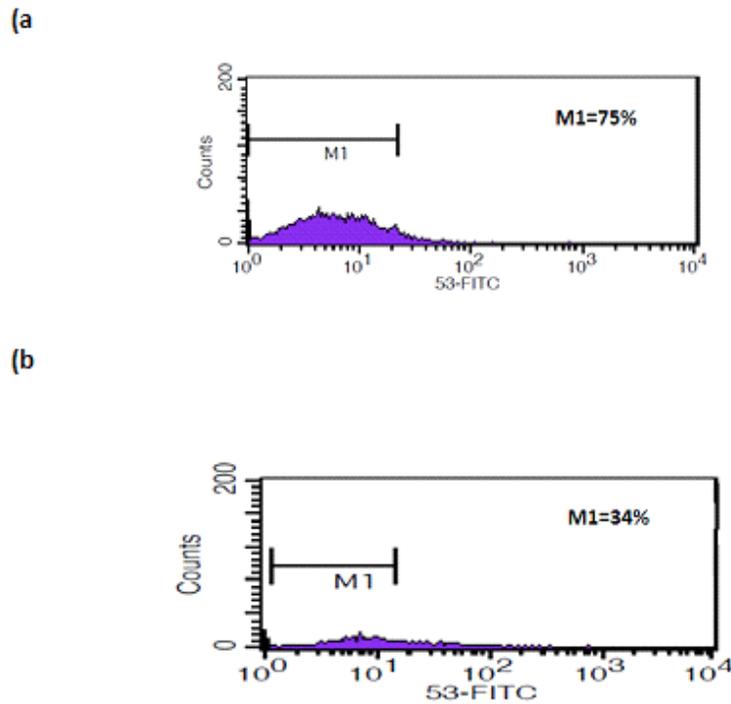
**Figure 3.** Sequencing results g.1386C>G (benign) missense mutation observed in astrocytoma (grade 4) patients compared to normal sequence.



**Figure 4** Detection of g.13835G>T (possibly damaging) missense mutation in astrocytoma (grade 4): a) Normal sequences. b) Mutant sequences.



**Figure 5.** Sequencing results g.13832A>G synonymous variation observed in astrocytoma (grade 4) patients compared to normal sequence: a) Normal sequences. b) Mutant sequences.



**Figure 6.** Flowcytometry histogram, Stained with fluorescent FITC dye, M1 represents the expression of p53 protein.

Above-mentioned nucleotide changes result in changes in amino acids as follows: lysine to glutamic acid (K291E), proline to arginine (P295R) and glutamic acid to aspartic acid respectively. Furthermore, flowcytometry analysis, using SPSS t-test, showed that the expression of p53 gene is significantly lower ( $p < 0.05$ ) in tumor samples compared to normal samples (Figure5). Statistical analysis using the t-test showed that the expression of p53 protein was significantly ( $p < 0.05$ ) lower in mutant samples compared to normal samples.

### **DISCUSSION**

In this study we showed that malignant mutations in exon 8 of the P53 gene are likely associated with high grade astrocytoma brain tumor. Three heterozygote missense mutations (g.13851A>G, g.1386C>G and g.13835G>T) at exon 8 of the p53 gene might have deleterious effects on its normal functioning, as they change amino acids in the DNA binding domain.

TP53 mutations usually occur late in tumorigenesis and are strongly associated with overall survival rate of patients. In high grade astrocytoma (especially grade 4) the disease affects more the brain hemispheres, and interestingly, brain hemispheres express

high levels of mutant p53 protein [12]. It might be one of the explanations that why P53 gene plays a pivotal role in development of malignant astrocytoma brain tumors. TP53 mutation and inactivation of P53 protein generally lead to uncontrolled cell growth, drug resistance and genetic instability, as it plays essential roles in cellular behaviors such as cell cycle arrest and cell death in response to DNA damaging agents [13]. This is important in gene therapy and detection of brain tumors in the early stages of the disease, in order to provide more efficient follow-up and more effective treatment.

### **CONFLIC OF INTEREST**

There is no conflict of interest among all of the authors of this manuscript.

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