p53 and p16\textsuperscript{INK4A} Mutations During the Progression of Glomus Tumor

Şefik GÜRAN,\textsuperscript{1} E Turgut TALİ\textsuperscript{2}

\textsuperscript{1}Department of Medical Biology and Genetics, Gülhane Medical Faculty, \textsuperscript{2}Department of Radiology, Gazi Medical Faculty, Ankara, Turkey

Glomus tumors are significantly rare tumors of carotid body. The great majority of these tumors are benign in character. Here we present two brothers with hereditary glomus jugulare tumor who had consanguineous parents. Radiotherapy was applied approximately 8 and 10 years ago for treatment in both cases. Eight years later, one of these cases came to our notice due to relapse. The mutation pattern of p53, p57\textsuperscript{KIP2}, p16\textsuperscript{INK4A} and p15\textsuperscript{INK4B} genes which have roles in the cell cycle, was analyzed in tumor samples obtained from the two affected cases in the initial phase and from one of these cases at relapse. The DNA sample obtained from the case in initial diagnosis phase revealed no p53, p57\textsuperscript{KIP2}, p16\textsuperscript{INK4A} or p15\textsuperscript{INK4B} mutation. He is still in remission phase. Despite the lack of p53, p57\textsuperscript{KIP2}, p16\textsuperscript{INK4A} and p15\textsuperscript{INK4B} mutation at initial diagnosis the tumor DNA of the other case in relapse revealed p53 codon 243 (ATG\textrightarrow{}ATC; met\textrightarrow{}ile) and p16 codon 97 (GAC\textrightarrow{}AAC; asp\textrightarrow{}asn) missense point mutations. No loss of heterozygosity in p53 and p16\textsuperscript{INK4A} was observed by microsatellite analysis of tumoral tissues in these cases. P53 and p16\textsuperscript{INK4A} mutations observed in relapse phase were in conserved regions of both genes. No previous reports have been published with these mutations in glomus tumor during progression. The mutation observed in this case may due to radiotherapy. In spite of this possibility, the missense point mutations in conserved region of p53 and p16\textsuperscript{INK4A} genes may indicate the role of p53 and p16\textsuperscript{INK4A} in tumor progression of glomus tumors.

Key words: glomus tumor, p53 and p16\textsuperscript{INK4A}, LOH

Introduction

Glomus tumor is a kind of tumor differentiated from nonchromaffin glomus chemoreceptor cells of the body. This tumor generally has benign character.\textsuperscript{1} The familial type shows autosomal dominant inheritance. Recently the affected gene in this tumor had been mapped to two distinct loci on chromosome 11q23.\textsuperscript{2,3} This tumor has a slow growing character and generally extends to the local sites. Metastases to the regional nodes and distant organs are extremely rare and late.\textsuperscript{2,4,5,6} Although benign in nature, the tumor can lead severe morbidity.\textsuperscript{3}

P53 is reported as the most frequently mutated gene in tumorigenesis.\textsuperscript{6} This tumor suppressor gene functions as a negative regulator of the cell cycle by stimulating cyclin dependent kinase inhibitors (CDKIs) which blocks entry of a cell from G1 to S.\textsuperscript{7-11} Regulation of the cell cycle is mediated by CDKIs, including p57\textsuperscript{KIP2}, p16\textsuperscript{INK4A} and p15\textsuperscript{INK4B} genes. The deletion or mutation of this locus on 9p21 therefore causes cell proliferation in a p53 dependent manner.\textsuperscript{16,17}

Received: Nov 27, 1998; accepted: Jan 19, 1999
Correspondence: Şefik GÜRAN, M.D., Ph.D., Department of Medical Biology and Genetics, Gülhane Medical Faculty, 06018, Etlik, Ankara, Turkey; Tel: +90 312 304 35 73, fax: +90 312 417 95 99; E-mail: gurans@gata.edu.tr
In this study, two previously reported brothers with the diagnosis of hereditary glomus jugulare tumor were analyzed for the mutation pattern of the p53, p57KIP2, p16INK4A and p15INK4B genes.

Case Reports

Case No 1 – MY is a 34 year-old man with the diagnosis of bilateral glomus jugulare tumor. Ten years ago in initial diagnosis phase, he had lateral neck masses on both sides. Radiologic studies showed enhancing masses on the right (5x8 cm), and left sides (4x8 cm). Biopsy material obtained from the external auditory canal revealed glomus tumor. The patient has been in remission phase for 10 years following radiotherapy.

Case No 2 – The brother of the proband (SY), a 28 year-old man, came to the hospital with similar signs and symptoms as his brother two years later. Radiologic examination of the tumors on the neck revealed bilateral enhancing masses of 2x2 cm on the right and 5x7 cm on the left. Biopsy material revealed glomus tumor. With radiotherapy, this case entered remission. Eight years later, he complained of hearing loss, tinnitus, vertigo and syncope. Radiologic examination showed 3x2 cm masses on the left jugular fossa. Biopsy material from the left jugular fossa revealed recurrent glomus tumor in this case.

Materials and Methods

Patient and samples

The peripheral blood DNA and the tumor DNA from paraffin sections of two affected cases were obtained for p53, p57KIP2, p16INK4A and p15INK4B gene analysis.

PCR-Single Strand Conformational Polymorphism (SSCP) analysis

The DNA’s for PCR-SSCP analyses were isolated by standard procedures from peripheral blood and paraffin sections. Exons 5 to 9 of the p53 gene, exons 1 and 2 of the p16INK4A and p15INK4B genes were amplified using primers designed from the published sequence. For p57KIP2, 6 sets of primers specific for the coding region of gene including exon 2 and 3 were used. PCR was performed according to standard methods. 50 ng of genomic DNA in a final volume of 25 µL was subjected to amplification in the present of 1µCi of [32P] dCTP (Dupont-NEN Research Product, Boston MA) with 1.5 mmol/L MgCl2, at annealing temperatures between 58°C and 60°C and for 30 cycles. Positive and negative controls were included in each reaction. PCR products were digested in 0.1% sodium dodecyl sulfate (SDS)/10 mmol/L EDTA and sequencing stop solution (Promega, Madison WI). For SSCP analysis, heat-denatured PCR products were electrophoresed on 6% nondenaturing polyacrylamide gels containing 10% glycerol. Gels were run at 8W for 12 to 15 hours at room temperature, dried and exposed to x-ray film at −70°C for 6 to 72 hours. PCR products greater than 250–300 bp (p15INK4B exon 1, exon 2 and p16INK4A exon 2) were digested with restriction enzymes prior to electrophoresis to increase the sensitivity of mutation detection (Bam HI for p15INK4B exon 1, Sma I for p15INK4B exon 2 and Kpn I for p16INK4A exon 2).

Direct DNA sequencing

All the fragments with anomalous SSCP were subsequently sequenced. First PCR products were purified with the Wizard PCR Preps DNA Purification System (Promega, Madison, WI) from 2% agarose gels. Sequences were obtained using the Promega fmol DNA Sequencing System.

Figure 1. a. SSCP results of p53 exon 7 in case 2 (Lane 1) which revealed gene alteration. Lane 1 had the DNA sample obtained in case 2 at recurrence. Lane 2 had the DNA sample obtained in case 2 at initial diagnosis. Lane 3 had the DNA sample obtained in case 1 at initial diagnosis. b. P53 codon 243 (ATG→ATC; met→ile) missense point mutation observed at recurrence in case 2. P = patient; N = normal
Kit (Promega, Madison, WI). The primers were first 5’ end-labeled using T4 polynucleotide kinase with [γ-32P]ATP (Dupont-NEN Research Product, Boston MA) and then a one step extension/termination reaction was performed according to the instructions of the manufacturer. The final products were denatured for 5 minutes at 95°C and 3 µl was analyzed in a denaturing 6% polyacrylamide, 8 M L urea sequencing gel for 2 or 3 hours at 55W. Both strands were sequenced for each DNA segment analyzed.

Loss of heterozygosity (LOH) analyses of p16 INK 4A gene

The LOH analyses of p16 INK 4A gene were performed on the tumor DNAs and the peripheral blood DNA of two affected cases. Four highly polymorphic short tandem repeats (STRs) or microsatellite markers specific for p16 INK 4A locus: D9S171, D9S942, D9S958, and IFNA were used. The same primers and conditions were used in our PCR reactions as described elsewhere: D9S171, D9S942, D9S958, and IFNA. In this panel, the protocol described by Piqueras et al was used in the LOH analyses of p16 INK 4A gene. The PCR fragments were electrophoresed on acrylamide gel. The fragments were visualized by using [32P]dCTP (Dupont-NEN Research Product, Boston MA). We made a comparison between the tumor DNA results and the peripheral blood DNA results for the LOH analyses in each case (Figure 3).

LOH analyses of P53 gene

The DNAs, purified from the tumor tissues and the peripheral blood of two affected cases were analyzed for LOH in p53. In LOH analyses, two polymorphic loci of the p53 gene were used: Restriction fragment length polymorphism (RFLP)-exon 4 and RFLP-intron 6. For the analyses of p53 gene, Barel at al’s protocol was applied.

Results

The tumor sample obtained from case 1 in initial diagnosis phase revealed no p53, p16 INK 4A, p15 INK 4B and p57KIP2 mutation. The tumor sample obtained from case 2 in initial diagnosis phase also revealed no p53, p16 INK 4A, p15 INK 4B and p57KIP2 mutation. In spite of these findings, the tumor material obtained from case 2 in relapse revealed p53 codon 243 (ATG → ATC; met → ile) and p16 INK 4A codon 97 missense point mutations (GAC → AAC; asp → asn) (Figure 2). No hereditary p53, p16 INK 4A, p15 INK 4B and p57KIP2 mutations were observed in the analyses of peripheral blood of two affected cases. We also analyzed the LOH findings in the tumor samples obtained from case 1 and 2 by using polymorphic markers specific for p53 and p16 INK 4A regions. No LOH in p53 and p16 INK 4A were observed in the analyses of tumor samples (Figure 3).

Discussion

Glomus tumors are very rare solid tumors which are generally benign in nature. A few cases of malignant glomus tumors have been reported with local invasive findings. Metastases of these tumors are exceedingly rare. Hereditary glomus tumor (MTM 168.000) which has autosomal dominant inheritance is a slowly progressive disorder causing benign tumor growth predominantly in the head and neck region. The gene responsible for hereditary glomus jugulare tumor had been mapped to two distinct loci on the long arm of chromosome 11 (11q23). In spite of these findings, molecular mechanisms involved in progression period are still unclear in glomus tumor. This manuscript is the first report of p53 and p16 INK 4A mutations, observed in relapse of hereditary glomus tumor.

Hereditary mutations of p53 and p16 INK 4A have been reported in some familial tumors. In our cases, no hereditary mutation was observed in the p53 and p16 INK 4A genes. LOH in mutated genes may be another possible gene alteration in tumor tissue. In the analyses of our two cases, no LOH was observed (Figure 3).
The case who had tumor material in relapse phase with p53 and p16(INK4A) mutations was treated with radiotherapy and accepted as in remission in the initial diagnosis phase. The effect of radiation on mutagenesis is well known. One report in the literature came to our notice with a malignant glomus tumor, possibly due to radiotherapy. In our case the mutations observed in the p53 and the p16(INK4A) genes may be due to radiation applied during the treatment.

The DNA obtained from the tumoral sample in recurrence phase revealed a novel p53 mutation in codon 243 (GAC→AAC; asp→asn) missense point mutation observed in case 2 is also a novel mutation in glomus jugulare tumor (Figure 2). This novel p16(INK4A) codon 94 mutation is in the second exon of the p16(INK4A) protein and it is in the conserved region of the p16(INK4A) gene. The mutated region of p16(INK4A) gene encodes the third ankyrin repeat of the p16(INK4A) protein. The mutation observed in the p16(INK4A) locus may have an effect on tumorogenesis via the INK4A-Cyclin D-ccd4-Rb pathway. In addition to this, the loss of inhibition effect of p53 gene on that pathway may also increase cell proliferation. On the other hand, the 9p21 region which encodes p16(INK4A) protein also encodes a second protein, p19ARF. The ARF-INK4A locus contains two unique first exons (α and β), which are spliced into common exons 2 and 3. Enforced expression of this protein (p19ARF) induces cell cycle arrest like p16(INK4A). The p16(INK4A) mutation observed in the second exon in ARF-INK4A locus and p53 mutation may involve regulation of the cell cycle on ARF-MDM2-p53 pathway.

As a result, finding the p53 and the p16(INK4A) mutations in one case with local invasion findings may indicate the importance of p53 mutations and p16(INK4A) in tumorigenesis of glomus tumor.

**Aknowledgements**

The authors are grateful to Dr. Zeki Güran from Oncology Hospital, Department of Radiotherapy, Ankara, Turkey for his kind help.

**References**