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Concurrent sequence variation of *TP53* and *TP73* genes in anaplastic astrocytoma

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ABSTRACT. Disruption or loss of tumor suppressor gene *TP53* is implicated in the development or progression of almost all different types of human malignancies. Other members of the p53 family have been identified. One member, p73, not only shares a high degree of similarity with p53 in its primary sequence, but also has similar functions. Like p53, p73 can bind to DNA and activate transcription. Using PCR-SSCP and gene sequencing, we analyzed the *TP53* and *TP73* genes in a case of a grade III anaplastic astrocytoma that progressed to glioblastoma. We found a deletion of AAG at position 595-597 of *TP53* (exon 6), resulting in the deletion of Glu 199 in the protein and a genomic polymorphism of *TP73*, identified as an A-to-G change, at position E8/+15 at intron 8 (IVS8-15A>G). The mutation found at exon 6 of the gene *TP53* could be associated with the rapid tumoral progression found in this case, since the mutated p53 may inactivate the wild-type p53 and the p73α protein, which was conserved here, leading to an increase in cellular instability.

Key words: Astrocytoma; *TP53* gene; *TP73* gene

INTRODUCTION

Gliomas, tumors of neuroectodermal origin, range from very "benign" astrocytomas to highly malignant glioblastoma multiforme, organized as an intermixed continuum rather than as discrete entities. Even low-grade gliomas may recur a few years later as a glioblastoma multiforme (Holland and Frei III, 1982). In astrocytic tumors, there is evidence that different genetic pathways lead to glioblastoma multiforme as a common end point. There appears to be two types of glioblastoma multiforme, denominated "primary" and "secondary" (Kleihues, 1998; Ohgaki and Kleihues, 2007).

Primary glioblastomas develop rapidly *de novo*, without clinical or histological evidence of a less malignant precursor lesion. They mainly affect the elderly and are genetically characterized by loss of heterozygosity 10q (70% of cases), *EGFR* amplification (36%), $p16^{INK4\alpha}$ deletion (31%), and *PTEN* mutations (25%). Secondary glioblastomas develop through progression from low-grade diffuse astrocytoma or anaplastic astrocytoma and manifest in younger patients. In the pathway to secondary glioblastoma, *TP53* mutations are the most frequent and earliest detectable genetic alterations, already present in 60% of precursor low-grade astrocytomas. Loss of heterozygosity of chromosome 17 and mutations in the gene frequently occur in low-grade astrocytomas and identical *TP53* mutations can be detected when these low-grade tumors progress to anaplastic astrocytomas and glioblastomas (Sidransky et al., 1992; Reifenberger et al., 1996). During progression to glioblastoma, additional mutations accumulate, including loss of heterozygosity 10q25-qter (~70%), which is the most frequent genetic alteration in both primary and secondary glioblastomas (Michael and Prados, 2000; Ohgaki and Kleihues, 2007).

The *TP53* gene family consists of three genes *TP53*, *TP63*, and *TP73*, which have non-overlapping functions in pivotal cellular processes, such as DNA synthesis and repair, growth arrest, apoptosis or senescence, genome stability, angiogenesis, development, and differentiation (Farmer et al., 1992; Levrero et al., 1999; Vogelstein et al., 2000; Vousden and Lu, 2002; Sbisá et al., 2007). These activities have been linked to the ability of p53 to bind to specific DNA sequences and activate transcription (Ko and Prives, 1996).

These genes encode sequence-specific nuclear transcription factors that recognize the same responsive element in their target genes (Bourdon et al., 1997).

The *TP53*, *TP73*, and *TP63* genes, through alternative splicing, can produce different isoforms, which differ in the C-terminal portion (De Laurenzi et al., 1999; Ueda et al., 1999; Bourdon et al., 2005).

It has been suggested that while the main role of *TP53* lies in the inhibition of tumor progression *TP73*, *TP63* appears to be much more strongly involved in development and differentiation (D'Erchia et al., 2003; Melino et al., 2003). The wild-type p53 protein is important in many cellular events, including upregulation of expression of the p21 protein and inhibition of cyclin-dependent kinases (Haas-Kogan et al., 1996; Gomez-Manzano et al., 1997). The *TP53* gene, found on chromosome 17p13, is frequently altered, often by point mutations and deletions in over 30 to 49% of cases, including lower-grade gliomas, suggesting an early, perhaps initiating event in malignant transformation (Watanabe et al., 1997).

The gene *TP73* not only shares a high degree of similarity with *TP53* in its primary sequence, but also seems to exhibit similar functions (Zhu et al., 1998; Arrowsmith, 1999). Given that they share amino acid sequence identity in the DNA-binding domain, *TP53* and *TP73* should have redundant functions in the regulation of gene expression. Indeed, p73 can activate TP53-regulated genes and suppress growth or induce apoptosis. Moreover, *TP53* and *TP73* are both induced by

DNA damage, albeit through distinct mechanisms. Because p53 and p73 are linked to different upstream pathways, this family of transcription factors might regulate a common set of genes in response to different extracellular signals and developmental cues (Levrero et al., 2000). We made an analysis of *TP53* and *TP73α* genes in a case of grade III anaplastic astrocytoma that had a further progression to glioblastoma.

SUBJECT AND METHODS

A 30-year-old male patient was operated for a WHO grade III anaplastic astrocytoma. One year later, there was a recurrence and the diagnosis was a WHO anaplastic glioblastoma (grade later III) and a WHO grade IV glioblastoma. High-molecular weight DNA was extracted from normal and tumor tissues by standard methods (Rey et al., 1992) and pathological tissues were classified by histological examination and graded according to WHO guidelines (Kleihues and Cavenee, 2000).

Genomic polymerase chain reaction (PCR) amplification was performed on exons 5, 6, 7, and 8-9 of the *TP53* gene and on exons 3, 4, 6, 7, 8, and 9 of the *TP73* gene. Table 1 shows the primers used for these genes. PCR conditions for *TP53* were 35 cycles of 95°C for 1 min, 57-61°C for 1 min and 72°C for 2 min, with a final extension of 8 min at 72°C. PCR conditions for *TP73* were 35 cycles of 94°C for 30 s, 57-61°C for 30 s and 72°C for 90 s, with a final extension of 7 min at 72°C. The PCR products were loaded onto 6-12% non-denaturing polyacrylamide gels (with or without 10% glycerol), electrophoresed and then silver stained (von Deimling et al., 1993). Samples displaying an altered PCR-SSCP (single-strand conformation polymorphism) pattern were re-amplified by PCR with the same set of primers, and the PCR products were sequenced with the ABI PRISM Big Dye Terminator Cycles Sequencing Kit (Perking Elmer, CA, USA). Each amplicon was sequenced bidirectionally.

Table 1. Primers used for the *TP53* and *TP73α* genes.

TP53		
Exon	Sequence (5'- 3')	Amplified fragment
5	F - 5' - TTCACTTGTGCCCTGACTT - 3' R - 5' - AACCAGCCCTGTCCGTCTC - 3'	269 bp
6	F - 5' - GGGCTGGTTGCCCAGGGT - 3' R - 5' - TAGTTGCAAACCAGACCTC - 3'	221 bp
7	F - 5' - GTGTTA(G)TCTCCTAGGTTG - 3' R - 5' - TGGCAAGTGGCTCCTGAC - 3'	193 bp
8-9	F - 5' - CCTTACTGCCTCTTGCTTC - 3' R - 5' - CTGGAAACTTTCCACTTGAT - 3'	384 bp
TP73α		
Exon	Sequence (5'- 3')	Amplified fragment
3	F - 5' - TGACACCCAAACTGGGGACTGA - 3' R - 5' - CCACTCCAGTCCTCTTGCAGA - 3	213 bp
4	F - 5' - GACGACTGACTGTGTTGTGTTTC - 3' R - 5' - CTCAGGGACTAGGGGAACTC - 3'	267 bp
6	F - 5' - ACCTCTATGCACCTCTCTGAAG - 3' R - 5' - GACCCGTACAGCTGACTGCA - 3'	213 bp
7	F - 5' - TTGGGGCTGCGTGCTGATGCTA - 3' R - 5' - CCTGCAGGTCTCCATGACAGCT - 3'	267 bp
8	F - 5' - CAGGGTTGAGCTCACAATTCTG - 3 R - 5' - TCCTCCCACACGCGTCCAGTT - 3	234 bp
9	F - 5' - ACCCTCTGGTCCTGCCTGCTCA - 3' R - 5' - ACGACAGAGGTGAGGCAGGTCT - 3'	192 bp

RESULTS

Using PCR-SSCP methods, we detected aberrantly migrated bands in two exons (one from *TP53* and one from *TP73*) (Figure 1A and C) and nucleotide sequence analysis revealed two types of changes (Table 2). The first was a polymorphism identified as an A-to-G change, at position E8/+15 of intron 8 (IVS8-15A>G) in the *TP73* gene (Figure 1B); another alteration was a deletion of AAG in the position 595-597 of *TP53* (Figure 1D), resulting in the deletion of Glu 199 in the protein.

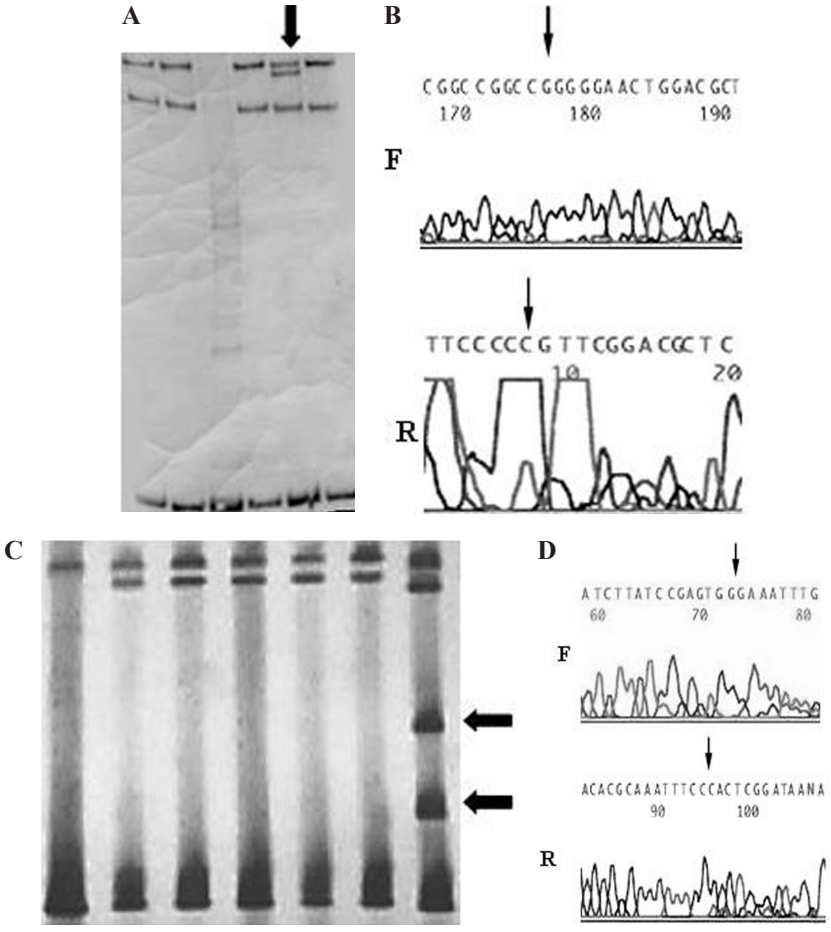

Figure 1. A. Exons 8-9 of the *TP73* gene. Arrow shows different mobility. **B.** Sequencing analysis shows (arrows) the genomic polymorphism of *TP73*, an A-to-G change, at position E8/+15 at intron 8 (IVS8-15A>G). **C.** Exon 6 of the *TP53* gene. Arrows show different motilities. **D.** Sequencing analysis shows (arrows) the deletion of AAG at position 595-597 del AAG/Glu in exon 6 of *TP53*. F = forward; R = reverse.

Table 2. Sequence variation identified in the *TP53* and *TP73* genes in anaplastic astrocytoma.

Gene	Exon	Nucleotide change	AA change
TP53	6	595-597 del AAG	Glu 199 del
TP73	8	IVS8-15A>G	-

DISCUSSION

TP73 expression is required for neurogenesis of specific neural structures, for pheromonal signaling and for normal fluid dynamics of cerebrospinal fluid. The *p73* gene has been considered to be a candidate tumor suppressor gene. Mutations in the *p73* gene are rare and have been reported in less than 2% of all cancers. *TP73* frequently undergoes loss of heterozygosity in breast and colon cancer, neuroblastoma, melanoma, and nonastrocytic brain tumors (Alonso et al., 2001a; Moll and Slade, 2004). It has been reported that there are at least 19 single nucleotide polymorphisms in *p73* gene (some in exons and others in introns), but none of them can induce an amino acid change (Alonso et al., 2001b; Ge et al., 2007). We found a polymorphism into the intron 8, when the base A was replaced by G, but without an amino acid change.

LOH 10q is most frequent in both primary and secondary glioblastomas. Epidermal growth factor receptor (*EGFR*) amplification, and *PTEN* mutations are genetic alterations typical of primary glioblastomas, whereas *TP53* mutations are early and frequent genetic alterations in the pathway leading to secondary glioblastomas. The *TP53* pathway plays a crucial role in the development of secondary glioblastomas. *TP53* mutations are the first detectable genetic alterations in two-thirds of precursor low-grade diffuse astrocytomas; this frequency is similar to that in anaplastic astrocytomas and secondary glioblastomas derived thereof (Ohgaki and Kleihues, 2007). Genetic instability due to the impaired ability of p53 to mediate DNA damage repair further facilitates the acquisition of new genetic abnormalities, leading to malignant progression of an astrocytoma into anaplastic astrocytoma. This is reflected by a high rate of *TP53* mutations (60-70%) in anaplastic astrocytomas and additional genetic abnormalities in the final progression process, leading to a secondary glioblastoma multiforme (Nozaki et al., 1999). In our case, we found an anaplastic astrocytoma that evolved to secondary glioblastoma, with deletion of an AGG codon at position 595-597, resulting in the deletion of Glu in the protein. This deletion occurred in a region of the protein domain and has not been reported before in human tumors. Similar mutations of *TP53* have been found to exert a dominant negative effect due to increased stability of the mutant protein compared to that of the wild-type protein (Finlay et al., 1989; Milner and Medcalf, 1991; Petitjean et al., 2007).

We suggest that the mutation found at exon 6 of the gene *TP53* is associated with the rapid tumoral progression found in our case, since the mutated p53 may inactivate the wild-type p53 and the p73α protein, which was conserved here, leading to an increase in cellular instability.

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