

# Influence of p53 Mutations on Prognosis of Patients with Glioblastoma

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**BACKGROUND.** The influence of *p53* mutations on the biology of astrocytic tumors is controversial. *p53* is thought to be inactivated in the early stage of gliomagenesis; however, what role its inactivation plays in the malignancy of gliomas remains unknown. To understand the significance of *p53* inactivation, the authors identified the locus of *p53* gene mutation in glioma samples at different stages of progression and studied the correlation between the mutation and clinical behavior.

**METHODS.** Samples from newly diagnosed gliomas, including pure and mixed astrocytomas, were analyzed for *p53* mutations using a yeast functional assay. To determine the locus of the gene mutations, DNA sequencing was performed.

**RESULTS.** The incidence of *p53* mutations was higher in anaplastic astrocytomas (AA, 48%) than glioblastomas (GBM, 31%). There was no significant difference in the average ages of GBM patients with and without *p53* mutations (54.9 years  $\pm$  2.3 and 53.2 years  $\pm$  4.6, respectively). In GBM patients, the mutation did not affect progression free survival or overall survival. Astrocytomas and GBM differed in the distribution of *p53* mutation loci.

**CONCLUSIONS.** The *p53* gene mutation does not markedly affect the survival of GBM patients. The difference in the location of *p53* mutations between AA and GBM suggests that in gliomas, the *p53* mutation may contribute not only to tumorigenesis (as an early event) but also to progression to malignancy (as a late event). *Cancer* 2002;95:249–57. © 2002 American Cancer Society.

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**KEYWORDS:** *p53*, astrocytoma, glioblastoma, yeast functional assay, prognosis, tumorigenesis.

**A**strocytic tumors are the most common adult neoplasms of the central nervous system. They have been classified into four clinico-pathologic groups: pilocytic- (PA), diffuse- (DA), and anaplastic astrocytoma (AA), and glioblastoma multiforme (GBM).<sup>1</sup> However, PA is thought to be clinically and genetically distinct from the other astrocytic tumors.<sup>2,3</sup> In addition, GBMs have been divided into two subgroups based on clinical and biologic features. Primary GBMs arise de novo, while secondary GBMs are the result of progression from lower grade astrocytomas. These tumors can now be characterized by their molecular genetic backgrounds. Some have suggested that primary and secondary GBMs are distinct disease entities that evolve via different genetic pathways.<sup>4,5</sup> Primary GBMs are characterized by amplification/overexpression of epidermal growth factor receptor (EGFR),<sup>6,7</sup> homozygous deletion of *p16*,<sup>8,9</sup> amplification/overexpression of murine double minute 2 (MDM2),<sup>10,11</sup> and the entire loss of chromosome 10.<sup>12</sup>

Secondary GBMs are characterized by functional loss of TP53 mainly caused by the gene mutations<sup>13,14</sup> and partial or complete loss of chromosome 10q.<sup>12</sup> As the *p53* gene mutation has been shown to occur in the early stage of progression to secondary GBM,<sup>14,15-19</sup> it can presumably be present in astrocytic tumors of different stages. Whether the mutation affects sensitivity to therapy and prognosis remains controversial.<sup>20-27</sup> To clarify whether *p53* mutations affect the clinical biology of these tumors, we explored the *p53* status of 123 surgical specimens using a well-established yeast functional assay. Mutations of the *p53* gene were identified by DNA sequencing.

## MATERIALS AND METHODS

### Patients and Tissue Specimens

The samples analyzed in the current study were obtained from the Department of Neurosurgery at Kumamoto University Hospital, Kumamoto, Japan, and its affiliated hospitals. They were from newly diagnosed, consecutive patients treated between 1995 and 2000. There were 73 males and 50 females ranging in age from 0 to 78 years (mean, 45 years). The patients and/or their legal guardians gave written informed consent for use of their specimens. Tumor specimens were obtained by surgical resection (including biopsy), quickly frozen, and kept at  $-80^{\circ}\text{C}$  until use. Formalin-fixed, paraffin-embedded specimens were subjected to histopathologic examination. Each specimen was classified according to established World Health Organization criteria.<sup>1</sup> The presence or absence of high cellularity, nuclear atypia, mitoses, microvascular proliferation, and necrosis were recorded. The presence of necrosis and/or microvascular proliferation was used as major criteria to distinguish between GBMs and AAs. There were 9 PAs, 15 DAs, 23 AAs, 55 supratentorial GBMs, 4 brain stem GBMs, 2 giant cell glioblastomas, 1 oligoastrocytoma, and 14 anaplastic oligoastrocytomas. All patients underwent surgical resection (including biopsy) with or without postoperative radiotherapy and/or nitrosourea-based chemotherapy. Most GBM patients younger than 70 years received both radio- and chemotherapy; older patients usually underwent only radiotherapy. To determine the extent of surgical resection we performed postoperative magnetic resonance imaging (MRI) study. Total resection was recorded when there were no residual lesions, subtotal resection when less than 10% of the preoperative mass remained, and partial resection when more than 10%

of the mass remained. For analysis, subtotal and partial resections were combined into the subtotal resection group. All patients were reevaluated after receiving initial adjuvant therapy; at periodical followup visit, MRI was performed. Clinical details, including the Karnofsky performance status (KPS) at the time of diagnosis, the extent of surgery, date of recurrence (or regrowth) on MRI, and date of death were recorded.

### mRNA Extraction and Reverse Transcriptase Polymerase Chain Reaction

The mRNA was extracted from the frozen tissue samples using the Quick Prep Micro mRNA Purification Kit (Amersham Pharmacia Biotech, Piscataway, NJ), and random hexamer-primed single-strand cDNA was synthesized using the SUPERScript Preamplification System (Life Technologies, Rockville, MD) according to the manufacturer's instructions. To amplify the *p53* cDNA, polymerase chain reaction (PCR) was performed in 25  $\mu\text{L}$  of reaction mixture containing 2.5  $\mu\text{L}$  of 10 X Pfu buffer (Stratagene, La Jolla, CA), 1.25 units of Pfu polymerase (Stratagene), 100 ng of each primer, 50  $\mu\text{M}$  of dNTPs, and 10% (vol/vol) dimethyl sulfoxide using a Thermal Cycler (Perkin-Elmer, Norwalk, CT) for 5 minutes at  $94^{\circ}\text{C}$ , for 35 cycles of 40 seconds at  $94^{\circ}\text{C}$ , 70 seconds at  $65^{\circ}\text{C}$ , 90 seconds at  $78^{\circ}\text{C}$ ; then for 8 minutes at  $78^{\circ}\text{C}$ . The *p53* specific primers for a 1 kb fragment encompassing codons 53-364 (exons 4-10) were P3 (5'-ATT TGA TGC TGT CCC CGG ACG ATA TTG AAsC-3', where s represents a phosphorothioate linkage) and P4 (5'-ACC CTT TTT GGA CTT CAG GTG GCT GGA GTsG-3').<sup>28</sup>

### Yeast Functional Assay

To investigate the *p53* gene status in various glioma subtypes, we performed a yeast functional assay as previously described.<sup>28</sup> Briefly, the *p53* PCR product and linearized *p53*-expression vector pSS16 were co-transfected into the reporter yeast strain yIG397, using the lithium acetate procedure. The transformed yeast cells were plated, incubated at  $30^{\circ}\text{C}$  for two days to generate colonies, and stored at  $4^{\circ}\text{C}$  overnight to develop color. At least 200 colonies were examined for each plate. When more than 15% of the colonies were red, we judged the sample positive for *p53* functional loss and proceeded to sequencing analysis.

### DNA Sequencing

To examine possible effects of *p53* mutations in gliomas, we performed DNA sequencing on samples

judged positive for the mutation. Eight red colonies from each positive plate were randomly picked and *p53* fragments were amplified by direct PCR with P3 and P4 primers, using rTth polymerase (PE Applied Biosystems, Foster City, CA). The PCR products were separated on an 0.8% agarose, then the DNA bands were excised and purified using a QIAquick Gel Extraction Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. Sequencing reactions were performed with P3 and P4 primers using a Big Dye Terminator Cycle Sequencing Kit (PE Applied Biosystems). The sequencing instrument was an ABI377 automated sequencer (PE Applied Biosystems).

### Statistical Analysis

Few patients with GBM, the most malignant glioma subtype, are treated successfully. To clarify whether *p53* gene mutations contribute to the malignancy of GBM, we analyzed the association between *p53* status and clinical parameters in adult patients with cerebral GBM. Progression free survival (PFS) and overall survival (OS) were calculated from the day of surgery to the day of recurrence or death. We used the student *t* test to evaluate the relationship between *p53* status and age and the chi-square test to determine the relationship between *p53* status and gender. The effect of different parameters, i.e., age (54 vs 55), gender, preoperative KPS (60 vs 70), extent of surgical resection (total vs subtotal/biopsy), and *p53* mutation status (intact vs mutated), on the Kaplan-Meier survival curve of PFS or OS was analyzed using the log rank (Mantel-Cox) test. The independent effect of each parameter on PFS and OS was analyzed using the multivariate Cox proportional hazards regression model. Also, to clarify the overall effect of *p53* status on clinical behavior regardless of tumor grade, we performed the same survival analyses on the entire patient population, including DA, AA, and GBM. All calculations were performed using Statview statistical software (Version 5.0; Abacus Concepts, Inc., Berkeley, CA). *P* values lower than 0.05 were considered significant.

## RESULTS

### *p53* Mutations and Statistical Analyses in GBM Patients

Clinical data and the results of mutation analysis are summarized in Table 1. *P53* mutations were present in 48% of AAs and 31% of supratentorial GBMs; they were less frequent, or absent, in the other subtypes. The ages of GBM patients with and without *p53* mutations were  $53.2 \pm 4.6$  and  $54.9 \pm 2.3$  years, respectively; there was no significant difference in

age or gender between these two groups. The median survival of GBM patients carrying the mutation was  $69.7 \pm 16.2$  weeks; it was  $71.2 \pm 3.4$  weeks for GBM patients with normal *p53*, and the difference was not statistically significant. *p53* mutations had no influence on PFS or OS not only in GBM (Fig. 1) but also in overall astrocytic tumors, including low grade and anaplastic astrocytomas (Fig. 2); OS was longer in younger patients ( $\leq 54$ ) and those with a higher KPS ( $70 \leq$ ;  $P = 0.003$  and  $P = 0.017$ , respectively; Tables 2 and 3).

### Sequencing Analysis

Of an overall 34 mutations, 28 (82%) were missense mutations; of these, 14 (50%) were located on hot spots. There was only one nonsense mutation (Patient 94, Table 1). Of the 28 missense mutations, 24 (86%) were transition mutations. There was no significance in these mutational characteristics in AA and GBM (Table 4). Of the 34 mutations, 4 (12%) were base deletions; one was a base insertion mutation. With respect to mutation location, 8 of 11 (73%) *p53* mutations in AA and 4 of 17 (24%) in GBM were located on exon 8 (Tables 1 and 4); the difference between AA and GBM was significant at  $P = 0.01$ .

## DISCUSSION

The tumor suppressor *p53* gene mutation is a genetic alteration often seen in astrocytic tumors, especially malignant gliomas, including AA and GBM. This mutation has been documented by single-strand conformational polymorphism study,<sup>21,22,26,29</sup> immunohistochemistry,<sup>13,15,18,30</sup> and yeast functional assays.<sup>28,31</sup>

The aim of the current study was to categorize gliomas genetically to promote the development of evidence based cancer therapies. If such genetics based treatment becomes available, a patient's *p53* status must be known. The yeast functional assay we employed can detect only loss-of-function mutations. However, it is simple and its results are reproducible,<sup>31,32</sup> and we found it to be as sensitive as other assays for the detection of mutations in the *p53* gene. We consider the yeast functional assay best suited for our current studies.

The influence of *p53* mutations in gliomas has already been discussed.<sup>1</sup> Tumor cells carrying *p53* mutations are resistant to apoptosis induced by DNA damage, while an overexpression of wild-type *p53* enhances the radiosensitivity of glioma cells.<sup>33-35</sup> However, the effect of *p53* mutations on the efficacy of radio- and chemosensitivity in patients with gliomas, especially GBMs, remains controversial. Some have reported that the *p53* status of GBM patients did not affect their survival<sup>22,29</sup> or

TABLE 1  
Genetic Status and Clinical Features of Patients with Glioma

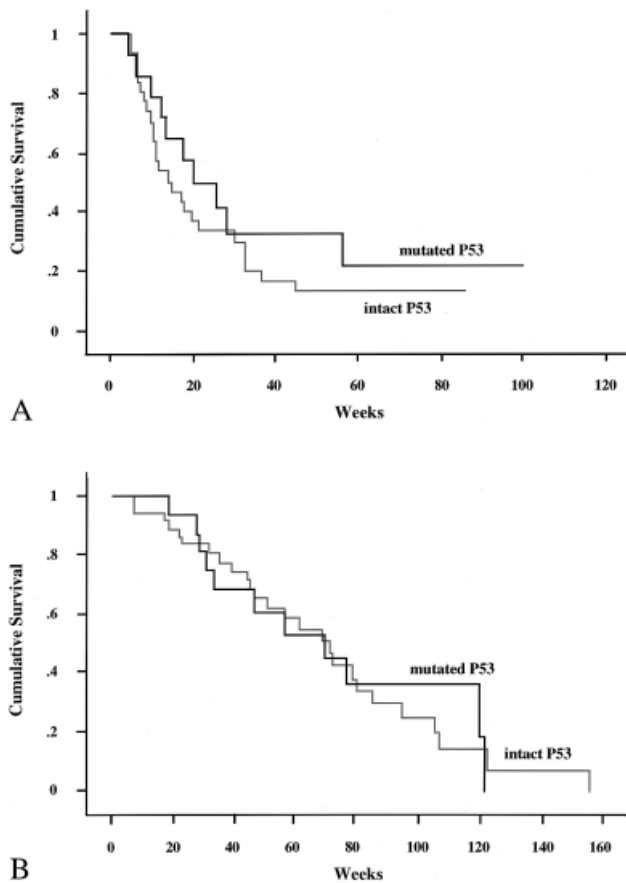
No.	Age in years	Gender	WHO Class	WHO Grade	p53 Mutation					KPS	Resect
					Mutation	Exon	Codon	Base change	AA change		
1	2	M	PA	1	N						
2	9	F	PA	1	N						
3	20	M	PA	1	N						
4	8	M	PA	1	N						
5	6	M	PA	1	N						
6	9	F	PA	1	N						
7	6	M	PA	1	N						
8	6	M	PA	1	N						
9	36	M	PA	1	N						
10	43	F	DA	2	N					90	total
11	48	M	DA	2	N					100	subtotal
12	62	M	DA	2	N					100	subtotal
13	4	M	DA	2	N					90	total
14	43	F	DA	2	N					100	subtotal
15	50	M	DA	2	N					80	subtotal
16	78	M	DA	2	N					80	biopsy
17	70	M	DA	2	N					90	biopsy
18	35	M	DA	2	N					80	subtotal
19	23	M	DA	2	N					80	biopsy
20	0	F	DA	2	N					90	total
21	41	F	DA	2	N					NR	subtotal
22	60	F	DA	2	P	6	220	tat to tgt	Y to C	100	total
23	62	M	DA	2	P	8	273	cgt to tgt	R to C	100	subtotal
24	39	M	DA	2	P	8	273	cgt to tgt	R to C	100	total
25	38	F	AA	3	N					100	NR
26	62	M	AA	3	N					70	biopsy
27	26	M	AA	3	N					70	subtotal
28	43	M	AA	3	N					NR	NR
29	43	M	AA	3	N					100	biopsy
30	72	M	AA	3	N					100	subtotal
31	50	F	AA	3	N					70	subtotal
32	68	F	AA	3	N					90	total
33	20	M	AA	3	N					70	subtotal
34	35	M	AA	3	N					100	subtotal
35	43	F	AA	3	N					100	total
36	26	M	AA	3	N					90	biopsy
37	54	F	AA	3	P	6	213	cga to caa	R to E	70	subtotal
38	41	M	AA	3	P	7	248	cgg to cag	R to Q	100	total
39	43	F	AA	3	P	7	261	agt to agg	S to R+ ins.	100	subtotal
40	40	M	AA	3	P	8	264	cta del.	L del.	100	subtotal
41	18	F	AA	3	P	8	273	cgt to cat	R to H	70	total
42	31	M	AA	3	P	8	273	cgt to tgt	R to C	100	subtotal
43	42	F	AA	3	P	8	273	cgt to tgt	R to C	90	biopsy
44	29	M	AA	3	P	8	273	cgt to cat	R to H	100	subtotal
45	56	F	AA	3	P	8	273	cgt to tgt	R to C	100	total
46	37	F	AA	3	P	8	280	aga to gga	R to G	20	subtotal
47	48	M	AA	3	P	8	280	aga to aaa	R to K	100	subtotal
48	54	M	GBM	4	N					70	total
49	69	F	GBM	4	N					80	subtotal
50	56	M	GBM	4	N					90	total
51	53	F	GBM	4	N					70	subtotal
52	54	F	GBM	4	N					50	subtotal
53	71	F	GBM	4	N					70	subtotal
54	75	F	GBM	4	N					60	total
55	66	F	GBM	4	N					100	subtotal
56	51	F	GBM	4	N					50	subtotal
57	63	F	GBM	4	N					100	subtotal
58	45	M	GBM	4	N					100	total
59	74	F	GBM	4	N					60	total
60	54	M	GBM	4	N					70	subtotal
61	29	F	GBM	4	N					70	biopsy
62	25	M	GBM	4	N					60	subtotal
63	59	M	GBM	4	N					90	biopsy

(continued)

TABLE 1  
(continued)

No.	Age in years	Gender	WHO Class	WHO Grade	p53 Mutation					KPS	Resect
					Mutation	Exon	Codon	Base change	AA change		
64	54	M	GBM	4	N					70	subtotal
65	51	F	GBM	4	N					90	subtotal
66	64	M	GBM	4	N					90	subtotal
67	38	F	GBM	4	N					10	subtotal
68	61	F	GBM	4	N					90	biopsy
69	47	M	GBM	4	N					90	total
70	53	M	GBM	4	N					60	subtotal
71	53	M	GBM	4	N					90	biopsy
72	45	M	GBM	4	N					70	total
73	72	M	GBM	4	N					100	subtotal
74	52	F	GBM	4	N					70	subtotal
75	40	M	GBM	4	N					80	total
76	68	M	GBM	4	N					60	subtotal
77	73	F	GBM	4	N					50	total
78	71	F	GBM	4	N					100	subtotal
79	48	M	GBM	4	N					100	total
80	64	F	GBM	4	N					70	total
81	65	M	GBM	4	N					90	subtotal
82	21	M	GBM	4	N					100	biopsy
83	61	M	GBM	4	N					70	total
84	20	F	GBM	4	N					60	total
85	69	M	GBM	4	N					80	subtotal
86	17	F	GBM	4	P	4	90 to 105	del.		100	subtotal
87	60	M	GBM	4	P	5	131	aac to del.	N to del.	70	subtotal
88	33	M	GBM	4	P	5	158	cgc to ctc	R to L	70	subtotal
89	71	M	GBM	4	P	5	175	cgc to cac	R to H	60	subtotal
90	71	M	GBM	4	P	5	179	cat to tat	H to Y	NR	subtotal
91	23	M	GBM	4	P	5	179	cat to ctt	H to L	50	biopsy
92	54	M	GBM	4	P	6	190	cct to ctt	P to L	60	total
93	23	F	GBM	4	P	6	195	atc to acc	I to T	100	subtotal
94	45	M	GBM	4	P	6	196	cga to tga	R to stop	100	total
95	47	M	GBM	4	P	7	245	ggc to agc	G to S	60	subtotal
96	77	F	GBM	4	P	7	246	atg to gtg	M to V	70	subtotal
97	74	M	GBM	4	P	7	248	cgg to tgg	R to W	80	subtotal
98	67	F	GBM	4	P	7	251	atc to ttc	I to F	90	total
99	56	F	GBM	4	P	8	267	cgg to tgg	R to W	80	subtotal
100	64	M	GBM	4	P	8	273	cgt to ggt	R to G	70	total
101	55	M	GBM	4	P	8	273	cgt to tgt	R to C	60	biopsy
102	69	F	GBM	4	P	8	282	cgg to tgg	R to W	80	subtotal
103	71	M	GBM, BS	4	N						
104	53	M	GBM, BS	4	N						
105	4	F	GBM, BS	4	N						
106	3	M	GBM, BS	4	P	8	277	tgt to ttt	C to F		
107	22	F	GGB	4	N						
108	32	F	GGB	4	P	5	148	cgg to cag	R to Q		
109	47	M	AOA	3	N						
110	42	F	AOA	3	N						
111	49	F	AOA	3	N						
112	51	F	AOA	3	N						
113	39	M	AOA	3	N						
114		M	AOA	3	N						
115	52	M	AOA	3	N						
116	43	M	AOA	3	N						
117	44	F	AOA	3	N						
118	37	M	AOA	4	N						
119	66	M	AOA	4	N						
120	65	F	AOA	4	N						
121	58	M	AOA	4	N						
122	45	F	AOA	3	P	5	131	aac del.	N del.		
123	1	M	OA	2	N						

WHO: World Health Organization; AA change: amino acid change; KPS: Karnofsky performance status; Resect: extent of resection; M: male; F: female; PA: pilocytic astrocytoma; DA: diffuse astrocytoma; AA: anaplastic astrocytoma; GBM: glioblastoma multiforme; BS: brain stem; AOA: anaplastic oligoastrocytoma; OA: oligoastrocytoma; GGB: giant cell glioblastoma; N: negative; P: positive; del: deletion; ins: insertion; stop: stop codon; NR: not recorded.

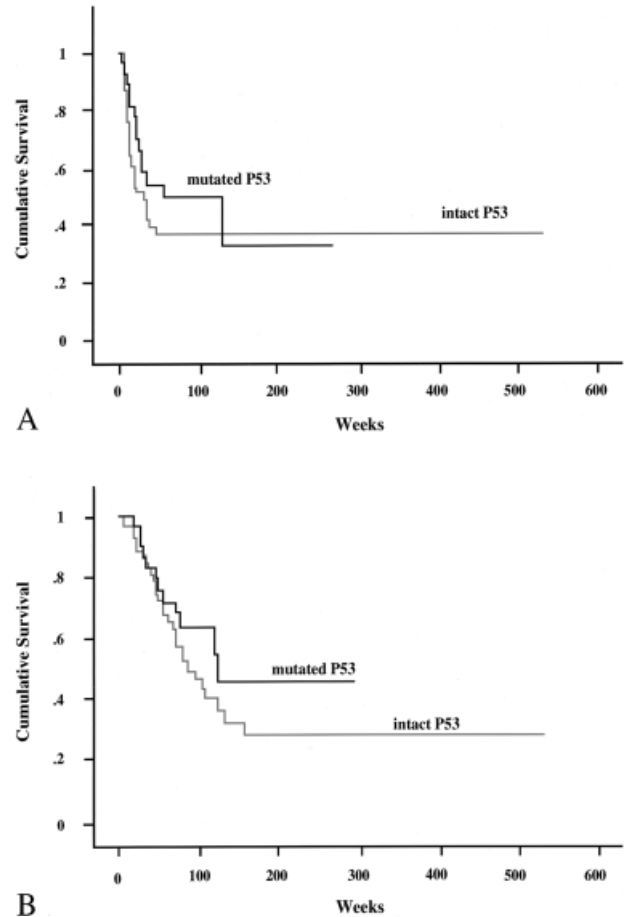


**FIGURE 1.** Kaplan-Meier A) progression free and B) overall survival curves in glioblastoma patients with mutated (black line, n = 17) or intact (gray line, n = 38) p53 (P > 0.05 in both A and B).

sensitivity to radiotherapy.<sup>21</sup> Others have shown that the presence of p53 gene mutations in GBM was associated with longer survival<sup>23,26</sup> and a better radiation response.<sup>23</sup>

The current data show that the time to tumor progression after surgery in patients receiving radiochemotherapy was not affected by the presence of p53 mutation. Therefore, the p53 gene mutation alone does not account for the radiochemoresistance of GBM. In fact, there is some evidence that p21 overexpression due to wild-type p53 overexpression results in radioresistance<sup>36,37</sup> and chemoresistance<sup>38</sup> in glioma, and that drug-resistance gene expressions render glioma cells chemoresistant.<sup>39</sup>

Diverse gene alterations are involved in glioblastoma progression. The MDM2 oncogene, whose product degrades and inactivates p53 protein,<sup>40,41</sup> is amplified and overexpressed in approximately 10% of GBMs, particularly primary GBMs with intact



**FIGURE 2.** Kaplan-Meier A) progression free and B) overall survival curves in patients with diffuse astrocytoma (n = 15), anaplastic astrocytoma (n = 23), and glioblastoma (n = 55) with mutated (n = 31) or intact (n = 62) p53 (P > 0.05 in both A and B).

p53.<sup>10</sup> Amplification of MDM2 leads to inhibition of the tumor-suppressive effects of p53. In gliomas, transcription of a short alternative splice variant of MDM2 is frequently observed.<sup>42,43</sup> It has been reported<sup>44</sup> that the variant lacks the ability to bind p53 protein for its degradation. This may be one explanation for the observation that in 30% of primary GBMs there is accumulation of wild-type p53 protein. The p53 mutation occurs early in the progression from low grade diffuse astrocytoma to glioblastoma.<sup>1</sup> We posit that in secondary GBMs that manifest the mutation, the mutation may be carried over from earlier stages in tumor progression. We found that in 73% of AAs and 24% of GBMs, the p53 mutation was localized to exon 8. This suggests that AAs containing the p53 mutation on exon 8 have a lower tendency for malignant progression. If this were not the case, GBMs could be expected to have

**TABLE 2**  
Univariate Analysis for Prognosis Factors in GBM

Characteristic	No. of cases	PFS		OS		
		Median ± SE (weeks)	P values	Median ± SE (weeks)	P values	
Age	54 ≥	27	14.4 ± 3.6	0.869	80.3 ± 7.7	0.018
	55 ≤	28	18.0 ± 2.6		46.3 ± 20.6	
Gender	Male	31	17.5 ± 4.7	0.863	61.4 ± 25.0	0.390
	Female	24	17.1 ± 6.1		71.3 ± 3.0	
KPS	60 ≥	15	9.9 ± 1.8	0.050	50.4 ± 10.1	0.055
	70 ≤	39	20.3 ± 2.5		78.3 ± 11.9	
	Not recorded	1				
Extent of resection p53 status	Subtotal/biopsy	37	14.1 ± 1.0	0.030	68.6 ± 12.4	0.639
	Total	18	44.9 ± 34.0		71.3 ± 5.0	
	Intact	38	14.4 ± 4.1	0.364	71.2 ± 3.4	0.925
	Mutated	17	20.3 ± 6.6		69.7 ± 16.2	

GBM: glioblastoma multiforme; PFS: progression free survival; OS: overall survival; SE: standard error; KPS: karnofsky performance score. P values lower than 0.05 were considered significant.

**TABLE 3**  
Multivariate Analysis for Prognosis Factors in GBM

Factor	PFS			OS		
	Hazard ratio	95% Confidence interval	P values	Hazard ratio	95% Confidence interval	P values
Age (54 ≥)	0.992	0.492–1.998	0.982	0.310	0.143–0.675	0.003
Gender (female)	0.726	0.338–1.559	0.412	0.652	0.305–1.397	0.271
KPS (70 ≤)	0.609	0.274–1.350	0.222	0.295	0.126–0.694	0.005
Extent of resection (total)	0.379	0.137–1.050	0.062	1.203	0.543–2.665	0.648
p53 status (mutated)	0.634	0.293–1.370	0.246	0.744	0.316–1.753	0.499

GBM: glioblastoma multiforme; PFS: progression free survival; OS: overall survival; KPS: Karnofsky performance score. P values lower than 0.05 were considered significant.

**TABLE 4**  
Profile of Overall Mutations in Glioma

WHO class	Missense (%)	Hot spot (%)	Transition (%)	Exon 8 (%)
AA	9/11 (82)	6/9 (67 )	9/9 (100)	8/11 (73)
GBM	14/17 (82)	6/14 (43)	11/14 (79)	4/17 (24)
others	5/6 (83 )	2/5 (40 )	4/5 (80 )	3/6 (50 )
total	28/34 (82)	14/28 (50)	24/28 (86)	15/34 (44)

WHO: World Health Organization; AA: anaplastic astrocytoma; GBM: glioblastoma multiforme.

acquired new p53 mutations on exons other than exon 8 as a later event in progression to GBM. On the other hand, if the propensity for malignant progression is indeed lower in AAs with the mutation on exon 8, one could expect a lower incidence of p53 mutations in GBMs. In fact, this was not the case in the current study, where no less than 31% of GBMs manifested the p53 mutation. In addition, the fact

that the rate of exon 8 mutation in DA was lower than in AA is inconsistent with the first hypothesis. We concluded that some of mutations in GBM were acquired de novo.

We also found that mixed gliomas rarely contained p53 mutations (Table 1). This suggests that in these tumors, the pathway for tumorigenesis or progression is different from that in pure astro-

cytomas. To classify gliomas genetically and to elucidate factors involved in their tumorigenesis and progression to malignancy, large scale studies must be performed that also address the status of other genes, such as *MDM2*, *p16*, *p14*, *EGFR*, and *PTEN*.

Mechanisms upstream and downstream from the *p53* tumor suppressive pathway are being revealed.<sup>40,41,45-48</sup> TP53 transactivates many target genes that are involved in various biologic functions. Each gene has its own *p53*-binding sequence in its promoter and is under the control of *p53* in a promoter specific manner. As we screened for the functional loss of *p53* by RGC, one of the binding sequences, we were unable to detect all possible mutations<sup>49</sup> and may have overlooked some mutations resulting in the loss of important unknown promoter activities. Studies are underway to determine whether such mechanisms are impaired in gliomas.

Although we found that in AA the *p53* mutation tended to be located on exon 8, this tendency may lack significance. The current finding may reflect promoter-selective transactivation. In breast carcinoma, *p53* mutations on exon 4 were associated with a particularly poor prognosis, while mutations on exons 6 and 7 and on hot spot regions were not, suggesting that different *p53* domains may affect patient survival differently.<sup>50</sup> Further investigations may yield information on whether this is also the case in patients with glioma.

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