Association of Mutant *TP53* with Alternative Lengthening of Telomeres and Favorable Prognosis in Glioma

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Abstract

The molecular basis for alternative lengthening of telomeres (ALT), a prognostic marker for glioma patients, remains unknown. We examined *TP53* status in relation to telomere maintenance mechanism (TMM) in 108 patients with glioblastoma multiforme and two patients with anaplastic astrocytoma from New Zealand and United Kingdom. Tumor samples were analyzed with respect to telomerase activity, telomere length, and ALT-associated promyelocytic leukemia nuclear bodies to determine their TMM. *TP53* mutation was analyzed by direct sequencing of coding exons 2 to 11. We found an association between *TP53* mutation and ALT mechanism and between wild-type *TP53* and telomerase and absence of a known TMM ($P < 0.0001$). We suggest that *TP53* deficiency plays a permissive role in the activation of ALT. (Cancer Res 2006; 66(13): 6473-6)

Introduction

The behavior of gliomas and hence the survival of patients with these tumors are determined by the molecular nature of the malignant cells. We have shown that, for patients with glioblastoma multiforme, telomere maintenance mechanisms (TMM) correlate with patient outcome (1). Patients whose tumors have an alternative lengthening of telomeres (ALT) mechanism (2) have a prolonged survival, whereas telomerase confers a poor survival. Interestingly, a significant fraction of glioblastoma multiformes has no known mechanism for telomere maintenance. Germline mutations in the *TP53* tumor suppressor gene are associated with increased risk of gliomas (3), and somatic mutations are found in a significant proportion of sporadic gliomas (4). The effect of these mutations on survival is equivocal (5–9), and the relationship of *TP53* status with TMM has not been studied previously. We correlated *TP53* status of gliomas with telomere maintenance and patient outcome. We show here for the first time that mutant *TP53* correlates strongly with the ALT mechanism and good prognosis, whereas, for patients with telomerase-positive tumors, mutant *TP53*, although rare, confers a worse prognosis.

Materials and Methods

Patient population. We obtained tissue samples from 110 patients recruited from Neurosurgical Units at Dunedin, Christchurch, Auckland (from year 2002-2005), Hull, and Sheffield (from year 1997-2002). The study was approved by institutional ethics committees, and all patients provided informed consent. The inclusion and exclusion criteria were the same as published previously (1). The histologic diagnoses were made by consultant neuropathologists at each center. All patients received surgical debulking followed by 60 Gy of fractionated radiotherapy using megavoltage X-ray. Patient age and survival were calculated from the time of first surgery. Patients were followed up until date of death or until Nov 10, 2005. Ninety-seven patients were included in the survival analysis (6 patients were excluded because the length of their follow-up was less than the reported median survival of 8 months; ref. 1; 7 patients were lost during follow-up.). This number of patients was considered appropriate for the survival analysis, as it was more than thrice greater than the minimum number of 30 events (10 events per degree of freedom; 3 degrees of freedom) required for the Cox proportional hazard regression model used in the study (10). The follow-up period for the six survivors in the survival analysis was 1,872, 900, 510, 450, 330, and 330 days. The median follow-up time for all patients was 243 days.

Telomere maintenance mechanism analysis. To determine the TMM of each sample, we measured telomerase activity and telomere length from frozen tissues using TeloTAGGG Telomerase PCR ELISA Plus kit (Roche Applied Science, Penzberg, Germany) and TeloTAGGG Telomere Length Assay kit (Roche Applied Science, Mannheim, Germany) as described previously (1). ALT status was confirmed for samples with long telomeres by staining for ALT-associated promyelocytic leukemia bodies (APB) on paraffin sections as described previously (11).

***TP53* mutation analysis.** In the United Kingdom samples, exons 2 to 11 of *TP53* were screened for mutations, including the intron-exon boundaries as described previously (12). For the samples from New Zealand, *TP53* exons together with the intron-exon boundaries were amplified by PCR; the products were annealed with products from the control cell line, A549, and then subjected to denaturing high-performance liquid chromatography done on a Wave DNA Fragment Analysis System (Transgenomics Limited, Crewe, United Kingdom; ref. 13). Samples with dissociation curves that differed from the control were sequenced. The likely effects of the identified *TP53* sequence changes were predicted using the Sorting Intolerant From Tolerant (SIFT) program and the "*TP53* mutations database" from the Universal Mutation Database.[8]

Statistical analysis. All the assays were done blinded to the study end point. χ^2 test and Fisher's exact test were done using GraphPad InState (version 3.05 for Macintosh, GraphPad Software, San Diego, CA). Survival was analyzed by the Kaplan-Meier method using GraphPad Prism version 4.00 for Macintosh (GraphPad Software).

Results

***TP53* mutations.** All 110 tumors studied were high-grade gliomas, including anaplastic astrocytoma in 2 (1.8%) patients

[8] http://www.umd.be:2072/index.shtml.

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Table 1. Clinical data for groups stratified by telomere maintenance mechanism phenotypes

TMM	Telomerase	None	ALT	Total	*P*
No. patients (%)	33 (30.0)	59 (53.6)	18 (16.4)	110 (100)	
Gender (*N* = 107)					
Male (%)	19 (61.3)*	32 (55.2)	9 (50.0)	60 (56.1)	0.7293
Female (%)	12 (38.7)	26 (44.8)	9 (50.0)	47 (43.9)	
Age (*N* = 106)					
Median, y	60	61	38	57	0.0001
Mean (SD), y	59.4 (13.5)	58.9 (12.14)	43.4 (17.0) †	56.2 (14.7)	
TP53 (*N* = 110)					
WT (%)	26 (78.8)	52 (88.1)	4 (22.2) †	82 (74.6)	0.0001
Mutant (%)	7 (21.2)	7 (11.9)	14 (77.8) †	28 (25.5)	

*Data are expressed as frequency (column percentage).
†Significant difference from other groups.

and glioblastoma multiforme in 108 (98.2%) patients. Overall, 18 of 110 (16.4%) gliomas had evidence of ALT. Thirty-three of 110 (30.0%) showed telomerase activity (Table 1). Fifty-nine (53.6%) tumors had no detectable TMM ("none"). *TP53* mutation was detected in 28 of 110 (25.5%) patients, which is consistent with another report (14). One patient had mutations in two exons (Table 2). Overall, 29 mutations were detected, of which 27 (93.1%) were missense mutations, 1 (3.5%) was a nonsense mutation, and 1 (3.5%) was a splicing mutation. Nineteen of 29 (65.5%) were transition mutations. Twenty-seven (93.1%) mutations were located

Table 2. Histologic and clinical characteristics of 28 high-grade gliomas with *TP53* mutations

Case	TMM	Histologic type	Age (y)	Gender	Mutation type	Structure domain	SIFT-normalized probabilities*	Survival (mo)
1	ALT	AA III	17	M	R248W	L3/DNA	0	30 †
2	ALT	GBM IV	38	F	Y126N	S2	0	32.8
3	ALT	GBM IV	57	F	Y126N	S2	0	6.2
4	ALT	GBM IV	38	F	V157F	S4	0	26.7
5	ALT	GBM IV	78	M	R158P	S4	0	22.4
6	ALT	GBM IV	25	F	D184Y	L2	0	62.4 †
7	ALT	GBM IV	34	M	Y220C	None	0	29
8	ALT	GBM IV	32	F	E221STOP	None	—	11.7
9	ALT	GBM IV	33	F	N239S	L3	0	11.6
10	ALT	GBM IV	36	F	R248Q	L3/DNA	0	22.5
11	ALT	GBM IV	55	F	R273C	DNA	0	22.8
12	ALT	GBM IV	42	M	R273H	DNA	0.05	17
13	ALT	GBM IV	20	M	R282W	H2	0	12.6
14	ALT	GBM IV	61	M	R282W	H2	0	4.9
15	None	GBM IV	69	F	P152A	None	0	11
16	None	GBM IV	64	F	P152T	None	0	20
17	None	GBM IV	41	M	Y220C	None	0	14.5
18	None	GBM IV	51	F	S227F	None	0	6
19	None	GBM IV	57	F	R273H	DNA	0.05	5.9
20	None	GBM IV	34	M	R273H	DNA	0.05	7.6
21	None	GBM IV	50	M	R273H	DNA	0.05	14.5
22	Telomerase	Astrocytoma	90	M	V272L	S10	0	7
23	Telomerase	GBM IV	64	F	Intron 4 splicing		—	7.7
24	Telomerase	GBM IV	60	F	L145P, E258A	S3, S9	0, 0	10
25	Telomerase	GBM IV	68	F	G244S	L3	0	—
26	Telomerase	GBM IV	66	M	R248W	L3/DNA	0	4.1
27	Telomerase	GBM IV	70	F	R282W	H2	0	2.2
28	Telomerase	GBM IV	24	M	R337C	None	0	1 †

Abbreviations: AA III, anaplastic astrocytoma grade III; GBM IV, glioblastoma multiforme grade IV.
*Values <0.05 are predicted to be deleterious; those ≥0.05 are predicted to be tolerated.
†Alive and well.

in the commonly mutated regions, exons 5 to 8. Outside exons 5 to 8, 1 mutation was found in exon 10, and a splicing mutation was found in intron 4. The most frequent mutation found in this study was R273H, which was detected in an ALT tumor and in three "none" tumors. Interestingly, we did not find any mutations in codons 175 or 245. Although SIFT results showed all mutations apart from R273H were deleterious, the R273H mutation has been reported to have defective activity toward promoters of various genes, including *BAX, MDM2*, and *WAF1* (15). We have also found that the R273H mutation fails to enhance activity of adenoviral major late promoter, the principal promoter regulating expression of late virus protein in the presence of E1A (16). The average age of patients with mutant *TP53* was significantly younger at diagnosis than those with wild-type (WT) *TP53* (49.1 $\pm$ 3.5 and 59.4 $\pm$ 1.4 years, respectively; $P = 0.0102$), which agrees with Louis et al. (17). In our study, *TP53* mutations were observed in 14 of 18 (77.8%) ALT tumors, which is significantly different from telomerase-positive tumors (7 of 36, 19.4%; $P < 0.0001$) and "none" tumors (7 of 59, 11.9%; $P < 0.0001$; Table 1). Mutant *TP53* and ALT were more likely to occur in younger patients, whereas WT *TP53*, telomerase, and "none" were more likely to occur in older patients.

ALT confers a survival advantage over and above mutant *TP53*. Previous reports on the prognostic value of *TP53* mutation in glioma are equivocal (5–9). In this study, *TP53* mutation alone affected the overall survival of glioblastoma multiforme patients (Fig. 1*A*). The median survival of glioblastoma multiforme patients carrying *TP53* mutation versus those with WT *TP53* was 12.2 and 7.6 months [ratio, 1.599; 95% confidence interval (95% CI), 0.9713-2.226; $P = 0.0032$]. The hazard ratio (HR) was 0.5202 (95% CI, 0.3323-0.8015; $P = 0.004$).

Previously, we have shown that patients with ALT-positive glioblastoma multiformes had a better survival than those with ALT-negative tumors (1). In this study, we found that ALT patients had better overall survival irrespective of their *TP53* status (22.4 and 20.65 months for mutant *TP53* and WT *TP53* each) than patients with telomerase/mutant *TP53*, telomerase/WT *TP53*, none/mutant *TP53*, and none/WT *TP53* (7, 6.7, 11, and 7.8 months, respectively; $P = 0.0008$; Fig. 1*B*). Patients with ALT-positive glioblastoma multiformes and WT *TP53* did as well as those with mutant *TP53* (Fig. 1*B*). It would be interesting to know if the *TP53* pathway is disturbed by other means in the ALT/WT *TP53* tumors. Moreover, ALT-negative/mutant *TP53* tumor patients did less well than ALT-positive/mutant *TP53* (7.6 and 22.4 months; $P = 0.0010$; Fig. 1*B*), showing that ALT seems to confer a survival advantage over and above mutant *TP53*. Thus, ALT per se has a positive effect on outcome and is not just a surrogate marker for *TP53* mutation. There is a trend that patients with none/mutant *TP53* had a better overall outcome than those with telomerase/mutant *TP53*, although it is not quite significant ($P = 0.0687$). This suggests that some of the "none" tumors are essentially different from telomerase tumors. *TP53* is no longer significant for outcome after adjusting for age, gender, and TMM ($P = 0.550$), but TMM is significant after adjusting for age, gender, and *TP53* status ($P = 0.006$ and 0.001 for the "none" and the telomerase groups compared with the ALT group; HR, 2.9258 and 3.7788).

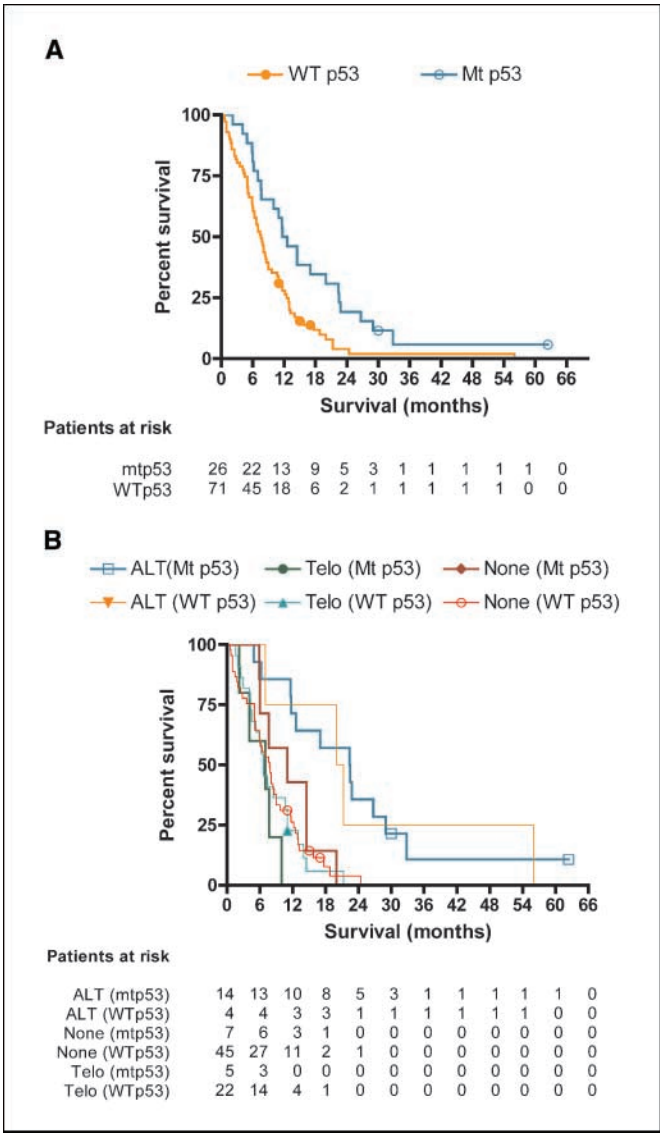

Figure 1. Kaplan-Meier analysis of patient survival with different (*A*) *TP53* status and (*B*) TMM and *TP53* status. Labels are patients who were still alive.

Discussion

Mutant *TP53* predisposes to ALT. Our data provide the first clinical evidence to support the notion that activation of ALT during tumorigenesis requires loss of normal *TP53* function. This is supported by the observation that >95% of ALT cell lines are impaired in the p53 pathway, either through *TP53* mutations or the expression of viral oncogenes (18, 19), and by *in vitro* studies, indicating that p53 inhibits DNA synthesis in ALT cells by a mechanism that seems to involve DNA binding and suppression of recombination (20). It therefore seems likely that *TP53* deficiency plays a permissive role in the activation of the ALT recombinational mechanism, so we speculate that occurrence of *TP53* mutation early in tumorigenesis predisposes to ALT. These findings may prompt similar studies in other forms of cancer for which ALT has been described (21).

We show that *TP53* mutation correlates with a less aggressive type of tumor, which is more likely to occur in young patients. We have shown previously that ALT is far and away the most powerful prognostic factor yet found for glioblastoma multiforme (1). Patients with an ALT-negative phenotype have poor prognosis irrespective of *TP53* status and/or young age. The prognostic implication of *TP53* status, which has been controversial for gliomas, can now be explained in terms of the association between mutant *TP53* and ALT and thus a more benign biology. Hence, the proportion of ALT patients

in a study population has a highly significant effect on whether *TP53* mutation correlates with good overall patient survival or not.

Our work shows that mutant *TP53* can no longer be regarded as a universal marker of poor survival. These data also suggest that targeting mutant *TP53* could form the basis of selective therapy for low-grade astrocytic lesions, which are likely to acquire an early *TP53* mutation (4) and the ALT mechanism of TMM (21) and to progress to secondary glioblastoma multiforme.

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