Advances in Brief

Selective Sensitivity to Radiation of Cerebral Glioblastomas Harboring p53 Mutations

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Abstract

Recent studies suggest that a balance may exist between the cell cycle arrest and apoptosis-inducing functions of the *p53* tumor suppressor gene. Adenoviral p21 transduction attenuates apoptosis, whereas deletion of the *p21* gene promotes it, and p21-null xenografts respond better than isogenic p21-wild type tumors to irradiation. Hence, the role of p53 in dictating the clinical response to radiotherapy and chemotherapy may be more complex than previously thought. We have analyzed survival and radiation response (regrowth-free period) of 42 patients with glioblastomas whose p53 status was determined by a sensitive yeast functional assay. Multivariate analysis revealed that p53 mutation is associated with longer survival ($P < 0.02$). Among 36 radiation-treated patients, the regrowth-free period after treatment was significantly longer for tumors with p53 mutations ($P < 0.0001$), and p53 mutation was the sole independent factor predictive of radiotherapeutic response ($P < 0.01$). Survival time after regrowth was independent of p53 status, suggesting that the difference in survival was related to the treatment rather than to the intrinsic aggressiveness of the tumor. Thus, in this Northern Japanese population, p53 mutation is a marker for better radiation response in glioblastomas, and this results in significantly longer survival.

Introduction

Mutation of the *p53* tumor suppressor gene confers striking radioresistance on xenografts in nude mice (1) and greatly accelerates malignant progression in genetically defined animal tumor models (2), but the adverse effect of p53 mutation in clinical studies rarely approaches the magnitude seen in animal studies (3). Clinically, those tumor types that rarely contain p53 mutations, such as testicular tumors and some childhood tumors, generally respond well to radiotherapy, whereas those that frequently contain p53 mutations, such as lung and colon tumors, respond poorly. The poor response of the latter, regardless of p53 status, may indicate that tumor types marred by frequent p53 mutation are marked by obligatory activation, followed by mutational inactivation, of the entire p53 pathway. If so, the prognostic value of p53 mutation in such tumors would reflect only the difference between inactivation of the pathway at the level of p53 itself and inactivation upstream or downstream of p53. There is evidence to suggest that the p53 pathway can be inactivated by mutation at the level of signaling [involving ATM (4), p19^{ARF} (5), and p33^{ING} (6)], feedback regulation (involving MDM2; Refs. 7 and 8), effector function (involving bax; Ref. 9), and regulators of effector function (involving bcl-2/bcl-X$_L$; Ref. 10).

Viewed in this way, the situation in which determining p53 status should provide the most valuable clinical information is in tumor types in which histological similarity conceals fundamental biological diversity. One such case may be malignant astrocytoma. Young patients are thought to undergo stepwise malignant progression punctuated by loss of p53 function (11, 12), in contrast with elderly patients who commonly develop *de novo* glioblastomas with intact *p53* genes but increased EGFR[2] expression (13). The interpretation that there are two distinct biological pathways of tumor formation is supported by the documented higher frequency of p53 mutations in anaplastic astrocytomas than in glioblastomas (14). Nevertheless, p53 mutation has been of uncertain prognostic value in ACGs (15–17), although it confers an unfavorable prognosis in low-grade astrocytomas (18, 19) and high-grade astrocytomas in children (20). Clinical factors reported to be of prognostic value for ACGs include age, extent of surgery, location of tumor, radiation therapy, and postoperative neurological status (KPS; Refs. 21–25), but patient survival rarely exceeds 5 years with even the most favorable combination of factors.

We previously used a yeast-based assay to detect transcriptionally inactive p53 mutants in glioblastomas occurring in a Northern Japanese population (14). With the rare exception of long-term survivors, survival times of most patients are now known, and striking differences in clinical outcome have emerged. The data suggest an unexpected inverse relationship between p53 mutation and response to radiotherapy and patient survival.

Patients and Methods

Patients. Three new cases are included in this study; the p53 status of the remainder was described by Tada *et al.* (14). All patients were operated on in the Hokkaido University Hospital or affiliated hospitals from July 1990 to December 1996 and diagnosed according to WHO guidelines (26); only histologically proven cases of glioblastoma multiforme were included in the study. They were principally consecutive cases, and their clinical outcomes were not investigated at the time of the yeast functional assay.

Yeast p53 Functional Assay and Sequencing. The yeast p53 functional assay was performed on the new cases (tumors 765, 797, and 806, giving 94%, 5.2% and 90% red colonies, respectively) as described previously (12, 14). Sequencing of plasmids recovered from yeast identified heterozygous 220TAT(Y)→TGT(C)/273CGT(R)→TGT(C) mutations in tumor 765 and a homozygous 286GAA(E)→AAA(K) mutation in tumor 806.

EGFR Expression. EGFR expression was determined by immunohistochemistry on formalin-fixed sections using Ab-4 anti-EGFR antibody from Oncogene Science (Uniondale, NY). Three researchers (R. M., M. T., and Y. Sh.) independently determined EGFR scores on blinded specimens according to the following criteria: 3, most tumor cells stained strongly; 2, some tumor cells stained strongly, with the remainder above background; 1, tumor cells gave weak staining only; 0, background staining only. Specimens with summed scores of 5 or more were considered positive.

[2] The abbreviations used are: EGFR, epidermal growth factor receptor; KPS, Karnofsky performance status; TTP, time to progression; PTD, progression to death; ACG, adult cerebral glioblastoma.

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Response to Radiation Therapy. Patients were irradiated after surgery using megavoltage X-rays from a linear accelerator, except for one patient who received neutron beam therapy. This patient was the long-term survivor in the wild-type p53 group but suffered significant neurological complications of the treatment. Magnetic resonance images taken sequentially (once a month) before and after radiation therapy were evaluated by two radiologists (R. O. and H. S.), who were not informed of p53 status, to assess the change in tumor size during radiotherapy and duration of tumor control after radiation therapy. TTP was defined as the interval in months from the end of radiotherapy to the date of magnetic resonance imaging-documented tumor regrowth. Disseminated tumors outside the radiation field were not taken in account. If continuous tumor growth was evident after radiotherapy, TTP was set at zero.

Log-Rank Tests for Survival and TTP. The effect of each factor on postoperative survival was tested, using a log-rank test (Cox-Mantel) on Kaplan-Meier actuarial survival curves, by comparison of the following group combinations: (a) glioblastomas with p53 mutations versus glioblastomas without p53 mutations, (b) glioblastomas with EGFR overexpression versus glioblastomas without EGFR overexpression, (c) males versus females, (d) patients younger than 50 years versus patients older than 50, (e) tumors of deep sites versus lobar tumors, (f) radical versus palliative surgery, and (g) postoperative KPS ≥80% versus KPS <80%. For location of tumors, those involving deep midline structures, such as the basal ganglia and brain stem, were classified as deep; others, involving frontal, parietal, temporal, and occipital lobes were classified as lobar. For the extent of surgery, removal of more than 90% of tumor bulk was classified as radical surgery; lesser removal was classified as palliative surgery. Analysis was done separately on all 42 patients and on a subset of 37 patients from which cases 32, 247, 611 (age below 20), 92 (spinal cord tumor), and 389 (immediate death after surgery) were excluded. The effect of each factor on TTP after irradiation was tested for the same groups, using the same method, excluding five patients (17, 241, 389, 611, 535) who did not receive radiation therapy.

Multivariate Analysis. The Cox proportional hazard model was applied for multivariate regression analyses to determine the contribution of individual factors to survival time and TTP.

Results

p53 mutation as a Determinant of Patient Survival. Of three new tumors tested by yeast assay in the present study, two were found to contain p53 mutations. Taken together with our previous results (14), mutations were found in 18 of 42 tumors (43%; Table 1). After a median follow-up period of 14.4 months, survival was analyzed relative to p53 status and other putative prognostic factors, including age, extent of surgery, postoperative KPS, location of tumor, and EGFR expression. Survival was analyzed separately for all 42 patients and for a subgroup of 37 adult patients with cerebral glioblastoma (exclusion criteria being age under 20, spinal cord tumor and immediate postoperative death). At the end of the observation period (December 31, 1997), there were nine survivors in the mutant p53 group, compared with only one in the wild-type group. Log-rank tests showed that younger age ($P < 0.04$), p53 mutation ($P < 0.001$),

Table 1 *Patient parameters*

Sample	Age	Sex	p53[a]	EGFR	Location	Surgery	KPS	Survival[b]	TTP[b]	PTD[b]	Remark[c]
17	34	F	wt	−	Lobar	Palliative	90	10.4			Rad (−)
32	12	F	mu	−	Lobar	Radical	50	38.9	6.2	29.3	
33	40	M	wt	−	Lobar	Palliative	70	77.5[d]	5.5	69.0	
47	49	M	wt	+	Lobar	Radical	90	16.0	6.9	6.9	
68	72	F	wt	NE[e]	Deep	Palliative	10	4.7	0.4	2.3	
75	70	F	wt	−	Lobar	Palliative	40	14.6	0.5	11.8	
87	65	F	wt	NE[e]	Deep	Radical	80	31.0	19.0	9.3	
89	23	M	mu	−	Deep	Palliative	80	5.4	3.6		
92	55	M	mu	NE[e]		Palliative	40	9.5	1.1	6.8	Spinal[f]
187	26	M	mu	−	Lobar	Radical	90	66.9[d]	61.4β		
197	66	M	wt	+	Lobar	Palliative	100	28.1	9.6	15.8	
200	66	M	wt	+	Lobar	Palliative	30	5.8	0.2	4.1	
214	44	M	mu	+	Lobar	Radical	100	19.5	8.0	9.8	
241	67	M	mu	+	Lobar	Palliative	40	25.6			Rad (−)
247	5	F	wt	−	Deep	Radical	100	9.2	3.8	3.7	
249	47	F	wt	NE	Lobar	Radical	30	7.4			Rad (−)
281	49	M	wt	−	Lobar	Radical	60	23.4	2.1	18.6	
322	55	M	wt	−	Lobar	Radical	90	15.9	8.2	5.5	
324	27	M	mu	+	Lobar	Radical	100	51.4[d]	44.4β		
333	31	M	mu	+	Lobar	Palliative	90	37.6	30.9	4.7	
334	49	F	mu	−	Lobar	Radical	90	49.8[d]	46.9β		
369	63	M	mu	−	Lobar	Radical	90	23.1	3.1	17.9	
370	52	M	wt	+	Lobar	Radical	90	10.8	1.6	7.4	
389	20	F	wt	−	Lobar	Palliative	0	0.2			Rad (−)
392	68	M	wt	−	Deep	Palliative	30	11.5	0.4	12.5	
438	66	M	wt	−	Deep	Palliative	60	9.2	1.7	5.5	
440	64	M	wt	−	Lobar	Palliative	100	15.0	2.3	10.2	
495	48	M	wt	−	Lobar	Palliative	100	14.1	0.0	12.3	
517	39	F	mu	+	Lobar	Palliative	80	37.7[d]	34.9β		
535	78	M	mu	+	Lobar	Radical	50	6.9			Rad (−)
544	27	M	mu	+	Lobar	Radical	100	35.7[d]	12.9	24.2	
564	68	M	wt	+	Lobar	Radical	60	15.1	1.2	11.4	
595	70	M	wt	−	Deep	Palliative	20	11.6	5.1	3.7	
611	16	F	mu	+	Lobar	Radical	40	4.7			Rad (−)
622	70	M	wt	−	Deep	Palliative	50	13.8	0.0	12.5	
635	74	M	wt	NE[e]	Lobar	Radical	80	9.2	2.8	4.9	
656	44	M	wt	+	Lobar	Radical	70	10.3	0.0	8.5	
677	63	F	mu	−	Lobar	Radical	100	27.1[d]	24.4β		
703	51	M	mu	+	Lobar	Palliative	70	19.7[d]	8.0	5.4	
765	22	M	mu	+	Lobar	Radical	90	15.9[d]	15.6β		
797	63	M	wt	−	Deep	Palliative	60	3.5	0.1	1.1	
806	62	F	mu	−	Lobar	Radical	60	14.2[d]	10.6β		

[a] wt, wild-type p53; mu, mutant p53.
[b] In months.
[c] Rad (−), patient did not receive radiotherapy.
[d] Alive at the end of the observation period; β stable tumor without regrowth at the end of the observation period.
[e] NE, not evaluated.
[f] Spinal, spinal cord tumor.

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Table 2 *Results of survival analyses by univariate log-rank test and multivariate Cox proportional hazard model*

Characteristics	No. of patients		Survival Mean ± SE (months)		Univariate *P*		Multivariate *P*	
	Total	ACG	Total	ACG	Total	ACG	Total	ACG
Age								
≤50	20	16	23.2 ± 3.4	25.1 ± 3.4				
>50	22	21	15.7 ± 1.9	16.0 ± 2.0	0.0388	0.0151	0.5437	0.2840
Sex								
Male	29	28	19.1 ± 2.2	19.4 ± 2.2				
Female	13	9	20.7 ± 4.5	21.0 ± 4.2	0.7836	0.3700	0.2319	0.8453
p53 status								
Wild-type	24	22	13.4 ± 1.6	14.2 ± 1.7				
Mutant	18	15	28.3 ± 3.4	29.7 ± 3.3	0.0010	0.0003	0.0053	0.0224
EGFR stain								
Negative	21	18	19.2 ± 3.0	15.9 ± 1.6				
Positive	16	15	22.4 ± 3.3	23.5 ± 3.3	0.5265	0.4395	0.1763	0.4809
Location								
Lobar	32	29	22.5 ± 2.4	22.9 ± 2.3				
Deep	9	8	11.1 ± 2.7	11.3 ± 3.1	0.0023	0.0019	0.0108	0.0297
Surgery								
Radical	22	19	22.5 ± 2.9	20.8 ± 2.2				
Palliative	20	18	16.5 ± 2.8	17.7 ± 2.9	0.1307	0.1607	0.5728	0.5676
Postoperative KPS								
≤70	21	17	15.0 ± 2.7	13.8 ± 1.9				
>80	21	20	23.8 ± 2.7	24.6 ± 2.7	0.0197	0.0187	0.4158	0.9192

superficial location of tumor ($P < 0.003$), and postoperative KPS score ($P < 0.02$) correlated with longer survival (Table 2; Fig. 1*a*). Because p53 mutation was associated with younger age (42 ± 20 years for mutant p53 *versus* 55 ± 17 years for wild-type p53, $P = 0.027$), multivariate Cox regression analysis was performed to determine the individual contribution of each factor. This identified absence of p53 mutation and deep tumor location as the only independent adverse risk factors (Table 2).

p53 Mutation as a Unique Determinant of Radiation Response. Response to radiation therapy was analyzed to see whether it could account for the difference in survival of patients with and without p53 mutations. There was no significant difference in radiation dose between p53 mutant and wild-type groups (60.5 *versus* 57.9 Gy; $P = 0.47$). Tumor response was assessed from sequential magnetic resonance scans. There was a significant correlation between TTP and longer survival (coefficient, $+0.719$; $P < 0.0001$). Log-rank tests showed that p53 mutation was the most significant factor contributing to longer tumor control ($P < 0.0001$), followed by postoperative KPS ($P < 0.0005$), location of tumor ($P < 0.018$), extent of surgery ($P < 0.023$), and age ($P < 0.027$; Table 3; Fig 1*b*). Cox multivariate regression analysis showed that p53 mutation and high postoperative KPS were independent determinants of TTP. In the more limited ACG

group, p53 mutation was the only significant factor ($P < 0.012$; Table 3). To further confirm that radiation is responsible for the longer survival, we analyzed time intervals between the detection of tumor regrowth and the death of the patient (PTD), because this should reflect the biological aggressiveness of the tumors. There was no significant difference in PTD for the ACG group between patients with and without p53 mutations (11.4 ± 2.9 *versus* 9.1 ± 1.1 months, respectively; $P = 0.3587$). Twenty-eight patients received chemotherapy, but this was not related to either survival ($P = 0.11$) or TTP ($P = 0.19$).

Discussion

This study illustrates the difficulty of extrapolating from simple experimental systems to complex human diseases. On the basis of studies in cultured cells (27) and xenografts in nude mice (1), it is widely assumed that p53 mutation confers radiation resistance in human cancer. Our results suggest that in glioblastomas, at least in Northern Japan, the reverse is true. In bladder cancer, an inverse relationship between p53 mutation and chemosensitivity has also been observed (28). Although this could be interpreted as showing that p53 mutation actually sensitizes cells to treatment with DNA-damaging
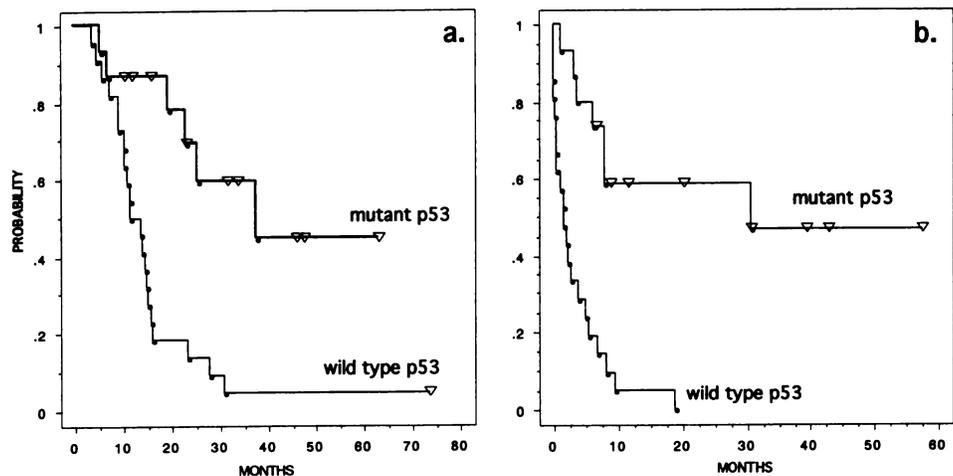

Fig. 1. *a*, actuarial survival curves for 37 ACG patients with and without p53 mutations, including patients alive (∇) and deceased (·) at the end of the observation period (December 31, 1997). *b*, curves for TTP after irradiation of the glioblastomas with and without p53 mutations, including patients with stable disease (∇) and patients without stable disease (·) at the end of the observation period (December 31, 1997).

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Table 3 *Results of radiation-response analyses by univariate log-rank test and multivariate Cox proportional hazard model*

Characteristics	No. of patients		TTP Mean ± SE (months)		Univariate P		Multivariate P	
	Total	ACG	Total	ACG	Total	ACG	Total	ACG
Age								
≤50	16	14	14.7 ± 3.4	16.0 ± 3.8				
>50	20	19	5.2 ± 1.5	5.4 ± 1.5	0.0269	0.0265	0.8295	0.8085
Sex								
Male	27	26	7.7 ± 2.0	8.0 ± 2.1				
Female	9	7	11.8 ± 3.1	13.7 ± 3.9	0.0653	0.0388	0.4776	0.2365
p53 status								
Wild-type	21	20	3.4 ± 1.0	3.4 ± 1.1				
Mutant	15	13	19.2 ± 3.5	21.6 ± 3.6	<0.0001	<0.0001	0.0027	0.0116
EGFR stain								
Negative	19	17	4.0 ± 0.7	3.8 ± 0.8				
Positive	13	13	13.2 ± 3.6	13.2 ± 3.6[a]	0.3389	0.4375	0.6181	0.7040
Location								
Lobar	26	25	12.5 ± 2.5	12.7 ± 2.6				
Deep	9	8	3.8 ± 2.0	3.8 ± 2.3	0.0144	0.0176	0.2778	0.6019
Surgery								
Radical	19	17	9.9 ± 1.8	10.5 ± 1.9				
Palliative	17	16	5.9 ± 2.4	6.2 ± 2.5	0.0185	0.0229	0.7806	0.9831
Postoperative KPS								
≤70	16	14	2.5 ± 0.7	2.4 ± 0.8				
>80	20	19	14.8 ± 2.9	15.4 ± 3.0	0.0004	0.0005	0.0318	0.0596

[a] The seeming difference in TTP between EGFR-positive and -negative cases reflects a difference in the middle part of the actuarial curves; as a whole, there was no significant difference.

agents, a more cautious interpretation is that retention of wild-type p53 is simply a marker for mutations elsewhere that confer an even greater resistance to therapy: the prognosis of patients with p53 mutations was better than that of patients with wild-type p53, but mean survival was still under 30 months. Regardless of the underlying biological mechanism, one important conclusion is that the clinical value of determining p53 status needs to be evaluated separately for each different type of tumor.

Several factors probably contributed to the clear-cut outcome of the present study despite its relatively small size. The short survival of patients with glioblastoma means that final measures of survival are available for most patients, at least in the wild-type p53 group. The method used to detect p53 mutations (29) is considerably more sensitive than most structural methods for detection of p53 mutations (14, 30, 31), and because it tests the function of the p53 protein, polymorphisms and silent mutations are scored correctly. It is conceivable that the yeast assay may have introduced some artifactual bias, but we consider this unlikely, based on our extensive use of the assay in other studies (12, 14, 29–31). A more serious caveat is that the Northern Japanese population may be unrepresentative of glioblastoma patients in general: the incidence of breast cancer in Hokkaido is lower than in Western populations, but the frequency of p53 mutations is 3-fold higher (81%) than has been reported elsewhere (32).

One intriguing possibility is that by imposing a p21-mediated G_1 arrest, p53 may actually protect glioblastomas from radiation-induced apoptosis. Cell fusion experiments have demonstrated that a balance exists between the cell cycle arrest and apoptotic functions of p53 (33). A role for p21 in this decision was confirmed by studies showing that deletion of both alleles of the *p21* gene sensitizes human colon carcinoma cells to ionizing radiation (34, 35), and adenoviral p21 transduction attenuates the apoptotic response of glioblastoma cell lines to subsequent p53 challenge (36). To explain the better outcome in p53-mutant glioblastomas, p53-mediated G_1 arrest would have to protect against p53-independent apoptosis (37). Interestingly, wild-type p53 activity has been shown to protect in exactly this way against apoptosis induced by chemotherapeutic drugs in fibroblasts (38). If this model is correct, suppression of the p21-dependent G_1 checkpoint should sensitize glioblastomas to irradiation. Overexpression of the *myc* oncogene can bypass p21-dependent arrest (39), and in appro-

priate conditions this provokes apoptosis (40). Thus, the G_1 checkpoint is a potential target for therapeutic manipulation in glioblastoma.

This study shows that p53 status can have predictive value in glioblastoma, but it offers little immediate hope to those patients with the worst prognosis, because wild-type *p53* gene therapy seems unlikely to benefit this group. For them, new approaches are required.

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