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p53 must be competent for transcriptional regulation to suppress tumor formation

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***In vitro* studies suggest that effective tumor suppression by p53 requires multiple domains to execute transcription-dependent and transcription-independent functions. We generated a mutant *p53* allele in mice, $p53^{W25QL26S}$ ($p53^{QS}$), containing an inactive transactivation domain to evaluate the importance of transactivation for p53-mediated tumor suppression. Recently, we discovered that the allele also contains a valine substitution for alanine at codon 135, which borders the DNA-binding domain. We found that $p53^{QSval135}$ bound to chromatin albeit less well than $p53^{QSala135}$, but both were equally deficient in transcriptional regulation, apoptosis induction in mouse embryo fibroblasts (MEFs), and suppression of tumor formation by E1A, Ha-Ras transformed MEFs. $p53^{QSval135}$ mice and *p53*-null mice exhibited identical tumor development kinetics and spectra in spontaneous and oncogene-initiated tumorigenicity assays, when tested in a homo- and heterozygous configuration. The $p53^{QSval135}$ allele did not have dominant negative functions and behaved as a null allele. Taken together, these data indicate that effective tumor suppression requires the transcriptional regulation function of p53, and they suggest that transactivation independent functions of p53 are unlikely to contribute significantly to tumor suppression *in vivo*.**
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Introduction

p53 signaling is functionally disabled in the majority of human cancers encompassing most tumor types (Nigro *et al*., 1989; Hollstein *et al*., 1991; Levine *et al*., 1991), and *p53* gene disruption in mice leads to spontaneous tumor development and early death (Donehower *et al*., 1992; Jacks *et al*., 1994). These data firmly establish *p53* as a tumor suppressor gene in vertebrates, but the mechanisms accounting for effective tumor suppression *in vivo* are still debated.

p53 is an unstable transcription factor, the abundance and activity of which are regulated predominantly by the E3 ubiquitin ligase MDM2 (Michael and Oren, 2003). The *MDM2* gene is activated by p53, creating a negative feedback loop to prevent precocious activation (see Wahl and Carr, 2001 for references). p53 is activated by diverse stresses, including DNA damage, hypoxia, aberrant oncogene expression, and ribonucleotide pool depletion, leading to cell cycle arrest, DNA repair, apoptosis, or cellular senescence (Wahl and Carr, 2001; Vousden and Lu, 2002). Post-translational modifications such as phosphorylation of N- and C-terminal serines and acetylation of the C-terminus (Appella and Anderson, 2001; Brooks and Gu, 2003), and MDM2 modifications that lead to accelerated MDM2 degradation, lead to p53 stabilization, and target gene activation (Stommel and Wahl, 2004).

p53-mediated transcriptional regulation and binding to target gene promoters correlate well with tumor suppression (Vogelstein and Kinzler, 1992; Prives, 1994). Furthermore, the biological functions of p53 most likely responsible for tumor suppression, cell cycle arrest, apoptosis, and DNA repair can be accounted for by its ability to activate or repress many target genes eliciting these effects (for recent reviews, see Vousden, 2000; Vousden and Lu, 2002; Wahl and Carr, 2001). On the other hand, some studies in cultured cells suggest that p53 can induce apoptosis independent of transcription (Caelles *et al*., 1994; Haupt *et al*., 1995; Haupt *et al*., 1997) through its interaction with mitochondria, direct activation of proapoptotic BH3-only proteins, or inactivation of antiapoptotic proteins such as Bcl-2 (Marchenko *et al*., 2000; Mihara *et al*., 2003; Chipuk *et al*., 2004). p53 may also limit genetic instability by directly contributing to repair of double-strand breaks and base damage (Mummenbrauer *et al*., 1996;

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Sturzbecher *et al*., 1996; Offer *et al*., 2001; Zhou *et al*., 2001; Rubbi and Milner, 2003).

Genetically engineered mice enable analysis of whether transcription-dependent and transcription-independent p53 functions and distinct p53 functional domains are required to suppress tumor formation in different tissues, or in response to various oncogenic stimuli. For example, the cell cycle arrest function of p53 is implicated in tumor suppression by the $p53^{R172P}$ mutant, which is defective in apoptosis induction but competent to induce p21 (Liu *et al*., 2004). Mice expressing $p53^{R172P}$ escape the early onset of lymphomas observed in *p53*-null mice, but tumors eventually arise that are largely diploid (Liu *et al*., 2004). By contrast, p53-dependent apoptosis is critical to limit the development of choroid plexus tumors initiated by inactivation of the pRb pathway (Symonds *et al*., 1994; Lu *et al*., 2001), or the induction of lymphomas initiated by *c-Myc* expression (Schmitt *et al*., 2002).

The contribution of the N-terminal p53 transactivation domain to tumor suppression also remains to be defined. Previous work showed that mutation of two adjacent highly conserved residues in the p53 N-terminus (L22QW23S in human; L25QW26S in mouse) severely disables transactivation measured *in vitro* (Lin *et al*., 1994) and in mice (Chao *et al*., 2000; Jimenez *et al*., 2000). Consequently, $p53^{L25QW26S}$ (referred to below as $p53^{QS}$) does not induce cell cycle arrest or apoptosis in response to DNA damage and other stresses in thymocytes or mouse embryo fibroblasts (MEFs) analysed *in vitro* (Chao *et al*., 2000; Jimenez *et al*., 2000). However, other studies implicate additional residues in the N-terminus as well as in the C-terminal regulatory domain in p53-mediated transcriptional control (Candau *et al*., 1997; Venot *et al*., 1999; Zhu *et al*., 1999; Barlev *et al*., 2001). In addition, a recent study showed that MEFs derived from the $p53^{QS}$ mice generated in this lab underwent apoptosis *in vitro* via a transcription-independent cytoplasmic mechanism (Chipuk *et al*., 2004). This is consistent with other work indicating transcription-independent mechanisms for p53-induced apoptosis involving p53–mitochondrial interactions (Mihara *et al*., 2003; Chipuk *et al*., 2004; Leu *et al*., 2004).

The initial description of the $p53^{QS}$ mice used mainly *in vitro* methods to study the biological effects of this mutation (Jimenez *et al*., 2000). The current report analyses the ability of $p53^{QS}$ to suppress tumor formation in several mouse models. We performed extensive tumor analyses to compare the $p53^{QS}$ and *p53*-null mice over a period of up to 2 years. Recently, we discovered that the parental targeting construct contains a mutation that generates valine substitution for alanine at codon 135 in $p53^{QS}$, which can engender conformational temperature sensitivity that can compromise p53 function. However, due to the elevated abundance of the stable $p53^{QSval}$ protein, the amount of $p53^{QSval}$ in the wild-type conformation *in vivo* exceeded that of wild-type p53 in normal cells. $p53^{QSval}$ bound to chromatin *in vivo*, but generally less well than wild-type p53 or $p53^{QSala}$. On the other hand, the $p53^{QSala}$ and $p53^{QSval}$ were equally deficient in target gene activation, apoptosis induction, and suppression of tumorigenicity in a nude mouse xenograft model. Importantly, $p53^{QSval}$ was equivalent to a *p53*-null allele with regard to suppression of spontaneous tumor development (tumor incidence, latency, and spectrum) and oncogene-induced tumor progression in a choroid plexus tumorigenesis model.

Results

L25QW26S ($p53^{QS}$) *mutation disables p53 function in mice*

The $p53^{QS}$ mutation was generated with a genomic targeting construct used previously to generate a *p53*-null allele (Donehower *et al*., 1992; Jimenez *et al*., 2000). A recent analysis of cDNA derived from $p53^{QS}$ MEFs revealed that alanine 135 was replaced by valine, which we showed to be present in the original targeting clone, and in the genomic $p53^{QS}$ allele. Conflicting reports have appeared on the functional consequences of val135. While this substitution can elicit temperature sensitivity (Milner and Medcalf, 1990; Martinez *et al*., 1991) and a mutant conformation that exhibits oncogenic activity in *in vitro* transformation assays (Michalovitz *et al*., 1990; Martinez *et al*., 1991), transgenic mouse strains exhibit different phenotypes ranging from modest tumor predisposition (Lavigueur *et al*., 1989) to premature senescence (Tyner *et al*., 2002).

The val135 mutation elicits a temperature sensitivity that can affect subcellular location and conformation (Milner and Medcalf, 1990) (Martinez *et al*., 1991). $p53^{QSval}$ is predominantly nuclear at both the permissive and nonpermissive temperatures (see Supplementary information, Figure S1A). We examined the conformation of p53 in thymus of $p53^{QSval}$ mice using discriminating antibodies. $p53^{QSval}$ samples exhibited both wild-type and mutant conformations, while the wild-type sample contained the expected predominance of wild-type conformer (Figure 1a). However, the greater stability and abundance of the $p53^{QSval}$ protein (Chao *et al*., 2000; Jimenez *et al*., 2000) yielded more wild-type conformer in $p53^{QSval}$ thymic tissue than in γ-irradiated $p53^{+/+}$ tissue (Figure 1a).

We next analysed the chromatin-binding activity of $p53^{QSval}$ protein in the thymus. Since neither $p53^{QSval}$ levels nor its binding to DNA changes upon stress (see below and Supplementary information, Figure S2), we examined $p53^{QSval}$ chromatin binding in unstressed $p53^{QSval/QSval}$ animals compared to chromatin binding of wild-type p53 in thymus of untreated or irradiated $p53^{+/+}$ mice. As expected, irradiation increased the abundance and chromatin binding of wild-type p53 to its response elements in the p21, Mdm2, Noxa, and Puma genes (Figure 1b). Although $p53^{QSval}$ protein is more abundant than wild-type p53 in unstressed cells, it bound at similar or somewhat lower levels than wild-type p53 did to these promoters *in vivo* (Figure 1b).

We compared the chromatin-binding activity and various biological functions of $p53^{QSval}$ and $p53^{QSala}$ (i.e.

wild type at codon 135) in MEF cells. We generated two independent cell lines, QSala-5 and QSala-6, that express $p53^{QSala}$ at levels similar to the endogenous $p53^{QSval}$ (Supplementary Figure S2) by infecting *p53*-null cells with lentivirus expressing $p53^{QSala}$. Again, $p53^{QSval}$ protein bound at similar or somewhat lower levels than wild-type did in unstressed MEFs (Figure 1c). By contrast, $p53^{QSala}$ bound better to the tested promoters, at similar levels as wild-type p53 did in adriamycin-treated cells (Figure 1c). Importantly, neither $p53^{QSala}$ nor $p53^{QSval}$ induced transcription of target genes such as p21, Mdm2, Noxa, and Puma, in the absence or presence of DNA damage (Figure 1d, Supplementary Figure S2). $p53^{QSval}$ was deficient in target gene activation and cell cycle arrest at the permissive or restrictive temperature (Supplementary Figure S1B and C), and neither $p53^{QSala}$ nor $p53^{QSval}$ induced apoptosis in E1A/ras-transformed MEFs in response to adriamycin or etoposide (Figure 2a). In total, the results show that while the val135 mutation does reduce chromatin binding *in vivo*, the $p53^{QSala}$ mutant is so deficient in function that the additional val135 mutation does not further compromise its ability to induce p53 target gene expression or to elicit cell cycle arrest or apoptotic functions.

p53 N-terminal transactivation domain is required for tumor suppression in an oncogene-driven xenograft assay

We next determined whether $p53^{QSval}$ and $p53^{QSala}$ retained some capacity to suppress tumor formation in E1A/ras-transformed MEFs. $p53^{+/+}$, *p53*-null, $p53^{QSval/QSval}$, and QSala cells were sequentially infected with retroviruses encoding E1A-12S and oncogenic Ha-ras (V12), the cells were injected into the flanks of athymic nude mice, and the animals monitored for tumor formation. In contrast to wild-type *p53* cells that failed to generate tumors, every mouse injected with $p53^{QSval}$- or $p53^{QSala}$-expressing cells developed tumors of various sizes, as did mice injected with $p53^{-/-}$ cells. Average tumor weights were not statistically different in these genotypes (Figure 2b). Figure 2c shows that most tumors generated by $p53^{QSval}$ and $p53^{QSala}$ clones expressed similar levels of the mutant p53 proteins, indicating that expression of these proteins does not prevent tumor growth. Taken together, these data indicate that perturbation of the N-terminal transactivation domain renders p53 inactive with respect to tumor suppression.

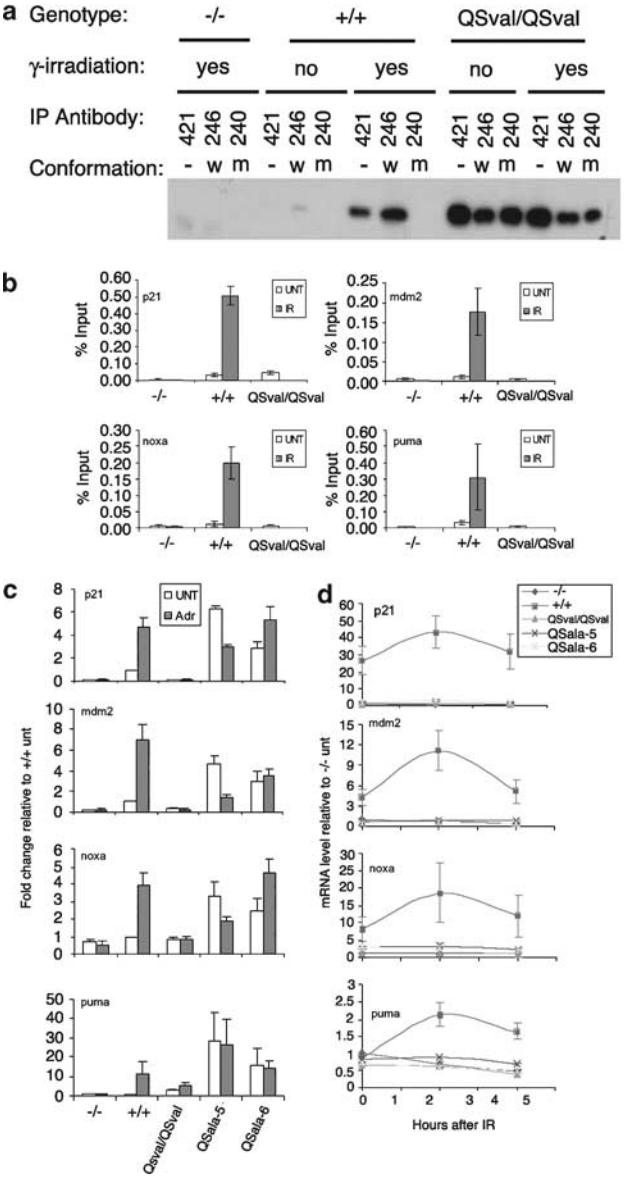

Figure 1 Characterization of $p53^{QSval}$ and $p53^{QSala}$ proteins. (**a**) Reactivity of $p53^{QSval}$ protein to conformation-specific antibodies. Equal amounts of protein (300 μg) extracted from thymus of $p53^{-/-}$, $p53^{QSval/QSval}$, and $p53^{+/+}$ mice (untreated or 2 h after 15 Gy γ-irradiation) were immunoprecipitated using conformation-specific antibodies pAb246 (wt conformation) and pAb240 (mutant conformation). pAb421 precipitated total p53 regardless of conformation. The precipitates were fractionated by SDS–PAGE and analysed by Western blotting using the polyclonal antibody CM-5. Only 1/6 of the precipitated protein from $p53^{QSval/QSval}$ thymus was loaded to compensate for the increased abundance of $p53^{QSval}$ protein. (**b**) ChIP assay of p53 responsive promoters in mouse thymus. Mice of the indicated genotypes were either untreated or analysed 2 h after 15 Gy γ-irradiation. Polyclonal antibody CM-5 was used to precipitate p53-bound DNA. The amount of p53 response elements bound was quantified by real-time PCR and normalized to input DNA. Error bars represent s.d. from two experiments. (**c**) ChIP assay of p53 responsive promoters in MEFs expressing indicated alleles. QSala-5 and QSala-6 are two independent clones that express $p53^{QSala}$ at levels similar to the endogenous $p53^{QSval}$ (see Supplementary Figure S2). Monoclonal antibody pAb421 was used to precipitate p53-bound chromatin from either untreated MEFs or MEFs treated with 0.2 μg/ml adriamycin for 12 h. Binding of the indicated p53 proteins to representative p53-responsive promoters were normalized to the amount of input DNA and compared to the binding of unstressed wild-type *p53*. Error bars represent s.d. from three independent experiments. Similar results were obtained when polyclonal antibody CM-5 was used for immunoprecipitation. (**d**) p53-dependent target gene activation after γ-irradiation of MEFs expressing indicated alleles. Total RNA was extracted and reverse transcribed using Superscipt III RT (Invitrogen). Transcription of p53 target genes was measured using quantitative PCR and normalized to ARP (acidic ribosomal phosphoprotein P0). The graph shows mRNA levels $\pm$ s.d. from two independent experiments. Primer sequences are available upon request

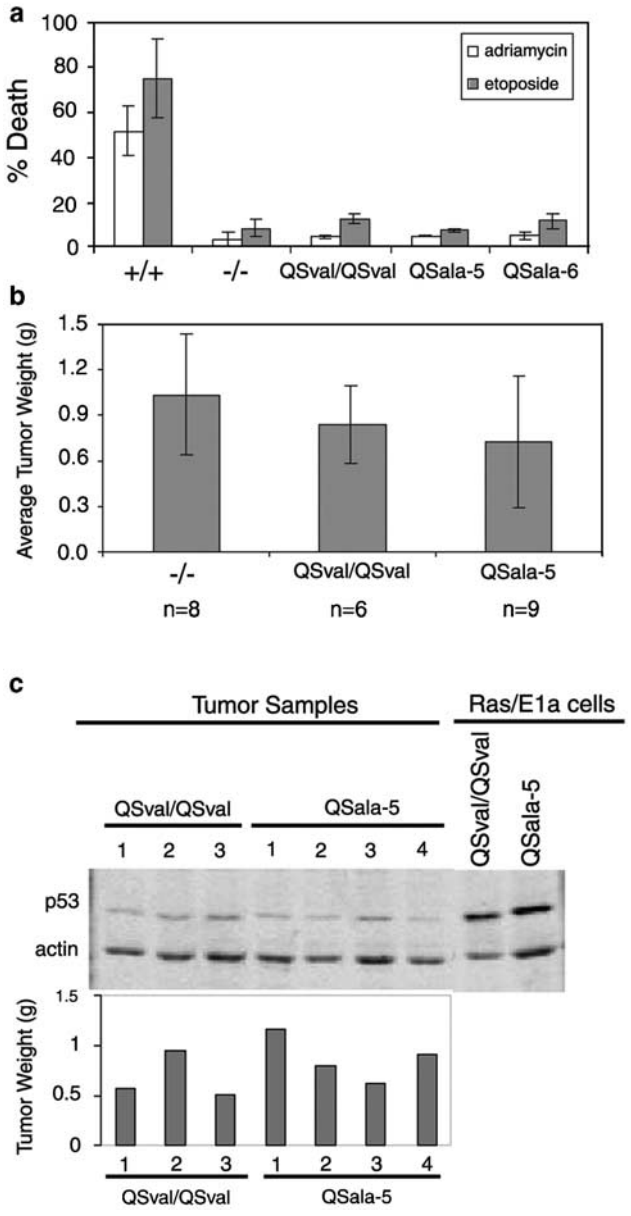

Figure 2 An intact p53 N-terminal transactivation domain is required for tumor suppression in an oncogene-driven xenograft assay in nude mice. (**a**) Apoptosis in oncogene-expressing MEFs. E1A- and Ras-expressing MEFs with the indicated *p53* alleles were treated with either 0.2 μg/ml adriamycin or 2.5 μM etoposide, and apoptosis was assessed 24 h following treatment by scoring annexin V-positive cells using flow cytometry. The percentage of cell death induced by the drugs was normalized to that of spontaneous death in corresponding untreated cells. The bar graph represents average cell death $\pm$ s.d. from at least two independent experiments. (**b**) Average size of tumors in athymic nude mice generated by oncogene-expressing MEFs with the specified *p53* alleles. E1A- and Ras-expressing MEFs were injected into the flanks of athymic nude mice. At 2 weeks after injection, the resulting tumors were isolated and weighed. The bar graph represents average tumor weight $\pm$ s.d. The total number of injected mice (*n*) for each cell type in two experiments is noted. (**c**) Comparison between tumor size and $p53^{QS}$ protein level. Proteins were extracted from individual tumor samples to assay for p53 protein expression by Western blot (upper panel) and results compared to tumor weight (lower panel). In parallel, p53 protein level was also measured in the E1A- and Ras-expressing cells used for mouse injection

Spontaneous tumor development occurs with identical kinetics in mice encoding $p53^{QSval}$ *and* p53*-null alleles*

We determined whether $p53^{QSval}$ suppresses tumor formation by analysing the spectrum and rates of spontaneous tumor development in heterozygous and homozygous mutant mice. We believe this is justified since $p53^{QSval}$ exhibits significant wild-type conformation in mouse tissues (see above), and we were unable to detect differences in the biological functions of $p53^{QSval}$ and $p53^{QSala}$ (Figures 1–2, Supplementary information, Figure S2). $p53^{+/QSval}$ mice were intercrossed to obtain $p53^{+/+}$, $p53^{+/QSval}$, and $p53^{QSval/QSval}$ offspring. The results were compared to data obtained from $p53^{+/-}$ and $p53^{-/-}$ mice of the same genetic background (Donehower *et al*., 1992; Jacks *et al*., 1994) and bred under similar conditions. Like the *p53*-null allele, $p53^{QSval}$ had a mild effect on embryonic development (see Supplementary Information).

p53-null mice developed life-threatening thymic lymphomas and/or sarcomas by 15–30 weeks of age. Survival measurements (Figure 3) revealed no statistical differences between mice that were homozygous for the $p53^{QSval}$ or null alleles ($P = 0.47$). The median survival time (t_{50}) for both $p53^{QSval/QSval}$ and $p53^{-/-}$ mice that developed tumors was approximately 150 days after birth (Figure 3 and Table 1a). Tumor penetrance was very high in both genotypes; 67/76 (88%) of the $p53^{QSval/QSval}$ and 26/31 (84%) of the $p53^{-/-}$ mice developed malignancies within the experimental timeframe. The survival data presented here agree well with previous studies (Venkatachalam *et al*., 1998).

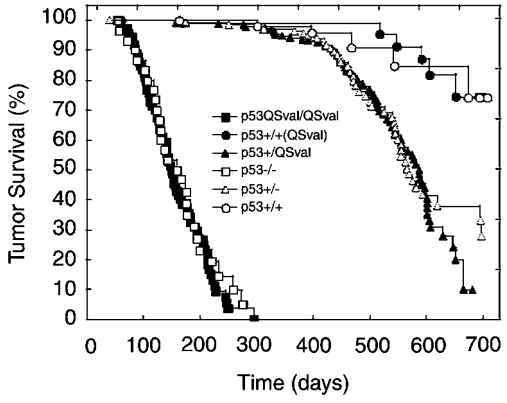

Figure 3 Kaplan–Meier cumulative survival analysis. (**a**) $p53^{QSval}$ and *p53*-null mice of $\geqslant$75% C57Bl/6 background were analysed, and the wild-type mice obtained from heterozygous crossings were analysed separately for the $p53^{QS}$ and *p53*-null mice. In total, 634 mice were analysed, including 76 $p53^{QSval/QSval}$, 201 $p53^{+/QSval}$, 122 $p53^{+/+}$ (out of the $p53^{+/QSval}$ breeding), 31 $p53^{-/-}$, 147 $p53^{+/-}$, and 57 $p53^{+/+}$ (out of the $p53^{+/-}$ breeding). When the tumor-specific survival of wild-type, heterozygous, and homozygous mice were compared within each mutation group, the differences in survival were all found to be highly significant ($p53^{QSval/QSval}$ *vs* $p53^{+/QSval}$ $P<0.001$; $p53^{+/QSval}$ *vs* $p53^{+/+}$ ($p53^{QSval}$) $P<0.001$; $p53^{-/-}$ *vs* $p53^{+/-}$ $P<0.001$; $p53^{+/-}$ *vs* $p53^{+/+}$ (null) $P<0.001$). However, $p53^{+/QSval}$ and $p53^{+/-}$ mice were very similar ($P = 0.53$), as were $p53^{QSval/QSval}$ and $p53^{-/-}$ mice ($P = 0.47$). The statistical analysis was limited to 15 months, but mice were followed for up to 2 years to allow for a more detailed observation of the heterozygotes

Although $p53^{QSval}$ and *p53*-null alleles were experimentally indistinguishable with respect to tumor development, the more stable but defective $p53^{QSval}$ protein could conceivably compromise the activity of the wild-type protein in heterozygotes by oligomerizing with wild-type p53. However, the survival curves for $p53^{+/QSval}$ and $p53^{+/-}$ mice were the same, within statistical confidence intervals ($P=0.53$), and 50% of $p53^{+/QSval}$ mice and $p53^{+/-}$ mice died with tumors by approximately 570 days after birth (Figures 3, 4 and Table 1a) (statistical analyses were limited to 15 months and data were censored to remove noncancer-related mortality). Therefore, there is no statistically significant tumor-related survival difference between animals carrying *p53*-null or $p53^{QSval}$ alleles in a homozygous or heterozygous configuration.

$p53^{QSval}$ *and* p53-*null alleles elicit the same tumor spectra*

Different tumor spectra in $p53^{QSval}$ and *p53*-null mice could occur if the $p53^{QSval}$ allele retains some function in specific cell types due to tumor suppressor functions unrelated to transactivation. Therefore, we autopsied animals of each genotype to ascertain the frequencies of specific tumor types. Table 1b shows that the tumor incidences were the same in animals with corresponding genotypes, regardless of whether they contained $p53^{QSval}$ or *p53*-null alleles. The incidence of lymphoma was 62 and 68%, respectively, in the homozygous $p53^{QSval}$ and *p53*-null mice and 12 and 10%, respectively, in the corresponding heterozygous mice. Sarcomas occurred equally often in all four analysed groups and the incidence of this tumor type was higher than the lymphoma incidence in heterozygotes (Table 1b), as noted in previous studies on *p53*-null mice (Donehower *et al*., 1992; Venkatachalam *et al*., 1998).

The lymphomas were subgrouped into lymphomas with thymic presentation and lymphomas with general presentation. Thymic lymphomas were more common in the homozygous mice, whereas lymphomas with general presentation showed a somewhat higher incidence in the heterozygous groups of mice (cf. Table 1b). Sarcomas were also subgrouped. Osteosarcomas were observed only in the heterozygous animals, while angiosarcomas were somewhat more common in the homozygous groups (Table 1b). Importantly, the incidence and latency of specific tumor types were similar in mice carrying either $p53^{QSval}$ or *p53*-null alleles (Figure 4a and b). Figure 5 reveals no systematic differences between $p53^{QSval}$ and *p53*-null mice with respect to the histopathology of the tumors within each subclass.

$p53^{QSval/QSval}$ mice also developed tumors other than lymphomas and sarcomas (Table 1c). By contrast, we did not observe these 'other' tumor types in homo-

Table 1a Median survival (days) specified for tumor types and genotype groups

Tumor type	$Trp53^{QSval/QSval}$	$Trp53^{-/-}$	$Trp53^{+/QSval}$	$Trp53^{+/-}$
All tumors	148 ($n=76$)	159 ($n=31$)	587 ($n=198$)	566 ($n=147$)
All lymphomas	141 ($n=47$)	140 ($n=21$)	507 ($n=25$)	515 ($n=15$)
Lymphoma, thymic pres.	127 ($n=28$)	118 ($n=10$)	458 ($n=7$)	NE
Lymphoma, general pres.	155 ($n=19$)	174 ($n=11$)	514 ($n=18$)	526 ($n=12$)
All sarcomas	154 ($n=15$)	127 ($n=5$)	478 ($n=30$)	484 ($n=23$)
Angiosarcoma	169 ($n=7$)	NE	NE	NE
Fibrosarcoma	132 ($n=4$)	NE	498 ($n=7$)	442 ($n=9$)
Osteosarcoma	NE	NE	453 ($n=15$)	547 ($n=13$)

NE: not evaluable, in groups with $\leqslant 3$ mice

Table 1b Incidence of tumor types and subtypes in the different genotype groups

Tumor type	$Trp53^{QSval/QSval}$	$Trp53^{-/-}$	$Trp53^{+/QSval}$	$Trp53^{+/-}$
Lymphoma, total	47 (62%)	21 (68%)	25 (12%)	15 (10%)
Thymic	28 (37%)	10 (32%)	7 (3%)	3 (2%)
Generalized	19 (25%)	11 (35%)	18 (9%)	12 (8%)
Sarcoma, total	15 (20%)	5 (16%)	31 (15%)	23 (16%)
Angio-	7 (9%)	1 (3%)	3 (1%)	1 (1%)
Fibro-	4 (5%)	1 (3%)	7 (3%)	9 (6%)
Osteo-	0 (0%)	0 (0%)	15 (7%)	13 (9%)
Other sarcomas	4 (5%)	3 (10%)	6 (3%)	0 (0%)
'Other' tumors	5 (7%)	0 (0%)	13 (6%)	7 (5%)
Tumors, total	67 (88%)	26 (84%)	69 (34%)	45 (31%)
No tumors	9	5	132	102
Mice, total	76	31	201	147

The tumor incidence patterns in $p53^{QSval/QSval}$ and $p53^{-/-}$ mice were identical ($P=0.45$), as were those in $p53^{+/QSval}$ and $p53^{+/-}$ mice ($P=0.80$). Incidence patterns were different when $p53^{QSval/QSval}$ and $p53^{+/QSval}$ mice ($P<0.001$) or when $p53^{-/-}$ and $p53^{+/-}$ mice ($P<0.001$) were compared

Table 1c All 'other' tumors occurring in the different genotype groups

Mouse #	*Tumor diagnosis*	*Age (months)*
Trp53$^{QSval/QSval}$		
54F4QS	Gonadal tumor	5.4
71F4QS	Anaplastic tumor under right cheek	3.0
#627	Malignant brain tumor (glioma)	5.6
#628	Malignant spinal cord tumor (glioma)	5.6
#663	Malignant testis tumor	1.9
Trp53$^{+/QSval}$		
23F4QS	Skin carcinoma	15.5
29F4QS	Squamous cell carcinoma of skin	5.2
46F4QS	Squamous cell carcinoma of skin	18.6
50F4QS	Squamous cell papilloma of skin	16.0
59F4QS	Liver adenocarcinoma	19.7
66F4QS	Skin carcinoma	17.9
74F4QS	Benign testis tumor (teratoma)	18.8
80F4QS	Anaplastic tumor in abdominal wall	16.4
10F3QS	Malignant brain tumor (glioma)	17.0
25F3QS	Squamous cell carcinoma of skin	16.1
#288	Anaplastic tumor, generalized	11.4
#302	Lung carcinoma	20.0
#329	Liver carcinoma	16.3
Trp53$^{+/-}$		
3F2KO	Adenocarcinoma in right arm pit	22.8
37F2KO	Cystic testis tumor	12.6
21F3KO	Skin carcinoma	12.1
15F3KO	Skin papilloma	18.5
#420	Liver carcinoma	15.4
#410	Carcinoma of salivary glands	14.7
#408	Gonadal tumor (teratocarcinoma)	15.8

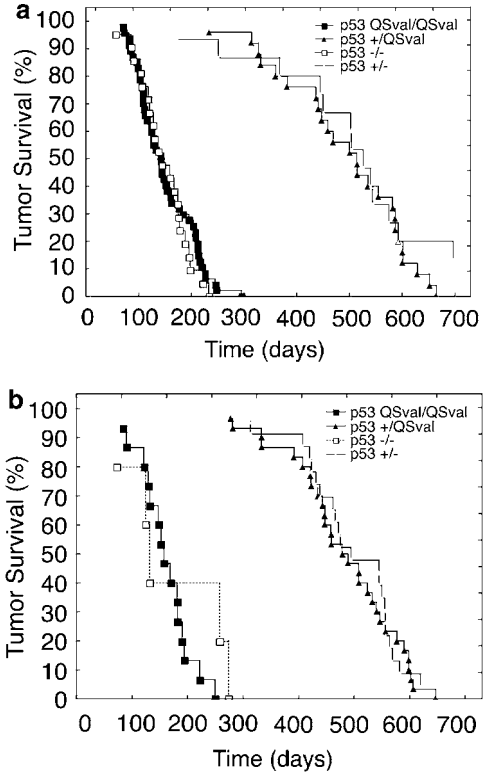

Figure 4 Latency of tumor development in homo- and heterozygous mice. Tumor-specific survival curves are shown for all lymphomas (**a**) and sarcomas (**b**) in homo- and heterozygotes. The latency of lymphoma development was shorter in homozygotes than in heterozygotes (**a**). This difference was statistically significant in *p53*-null mice, while there was a tendency for such a difference in *p53*^{QSval} mice (*p53*$^{-/-}$ *vs p53*$^{+/-}$, $P=0.015$; *p53*$^{QSval/QSval}$ *vs p53*$^{+/QSval}$, $P=0.09$). Notably, the longer latency in heterozygotes was associated with a higher fraction of lymphomas with general presentation (cf. Table 1a and b). Similarly, the latency of sarcoma development was shorter in homozygotes than in heterozygotes (**b**) even though the overall sarcoma incidence was approximately the same in hetero- and homozygotes. However, the sarcoma subtype spectrum was different in homo- and heterozygotes (cf. Table 1b and Results)

zygous *p53*-null mice. The low number of mice developing 'other' tumors does not allow attribution of the apparent difference to the *p53*^{QSval} allele, the small *p53*$^{-/-}$ cohort, and/or an influence of the 129/SvJae background (<25%) in the *p53*^{QSval} mice. No homozygous *p53*^{QSval} mice developed epithelial tumors, same as what is commonly observed for *p53*-null mice (Donehower *et al*., 1992, Jacks *et al*., 1994). Both groups of heterozygous mice developed epithelial tumors, mostly at older age. In summary, in considering all parameters of tumor development, the *p53*^{QSval} allele behaves as a null allele.

Tumor LOH analysis indicates that p53^{QSval} *behaves as a null allele*

Tumors developing in *p53*$^{+/-}$ animals frequently retain one functional wild-type allele (Venkatachalam *et al*., 1998, 2001). Although the data reported above indicate that the stable p53^{QSval} protein does not act in a dominant negative fashion, it is possible that it might exhibit some residual function relevant to tumor suppression. Were this true, one might expect a higher frequency of wild-type allele loss. We therefore determined the frequency with which the wild-type allele was lost from tumors in *p53*$^{+/-}$ and *p53*$^{+/QSval}$ mice by allele-specific PCR (Table 2, Supplementary information, Figure S3). Five of 18 (28%) tumors from *p53*$^{+/QSval}$ mice and 2/9 (22%) tumors in *p53*$^{+/-}$ mice lost the wild-type allele. This frequency is low compared to previous reports (Venkatachalam *et al*., 1998) where approximately 50% of the *p53*$^{+/-}$ tumors lost the wild-type allele. This discrepancy could be due to the small number of tumors investigated here and/or to methodological differences. Nonetheless, we found no difference in allele loss whether the mutant allele was null or *p53*^{QSval}. We conclude that the *p53*^{QSval} allele is equivalent to a null allele with regard to retention of the wild-type allele during spontaneous tumor development in heterozygotes.

Activity of the p53^{QSval} *allele in suppressing tumor progression*

p53 inactivation is often associated with tumor progression involving activated oncogenes. Furthermore, most tumors in humans develop from compromised cells in a

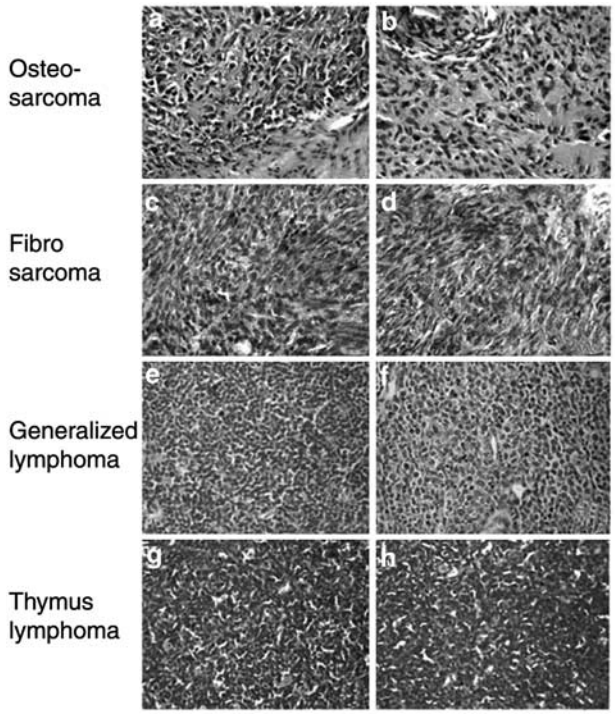

Figure 5 Microscopic morphology of tumors in the $p53^{QSval}$ and *p53*-null mice. There were no systematic differences between $p53^{QSval}$ and null mice with regard to the histopathological appearance of tumors for each tumor type. For example, among sarcomas, high and low differentiated fibrosarcomas were found in both groups as were histiocytic (MFH, malignant fibrous histiocytoma-like) tumors. Tumor morphology is exemplified by tumors occurring in $p53^{+/-}$ (**a**, **c**, **e**); $p53^{+/QSval}$ (**b**, **d**, **f**); $p53^{-/-}$ (**g**); and $p53^{QSval/QSval}$ (**h**) mice. The diagnoses are osteosarcoma (**a**, **b**); fibrosarcoma (low differentiated) (**c**, **d**); lymphoma (with general presentation) (**e**, **f**); and lymphoma (with thymic presentation) (**g**, **h**). Photographs were obtained with a Leica microscope, 20 × objective, and digital camera

context of genetically normal cells. Thus, we examined whether $p53^{QSval}$ retained function as a tumor suppressor in such a context. In epithelial cells of the choroid plexus (CP; in brain), as in most epithelia in the mouse, *p53* inactivation does not predispose to tumorigenesis (Donehower *et al*., 1992; Jacks *et al*., 1994). However, inactivation of pRb and related proteins, p107 and p130, by cell-specific expression of T_{121} (a truncated SV40 T-antigen fragment) changes the selective milieu. In TgT_{121} mice, this normally nondividing epithelium becomes hyperproliferative, resulting in activation of a p53-dependent apoptotic response. In a *p53* nullizygous background, apoptosis is reduced by 85% and tumors grow at an accelerated rate, reducing survival time by seven-fold (Symonds *et al*., 1994). Thus, this system provides a sensitive quantitative assay for p53 tumor suppressor function *in vivo*. Furthermore, several p53 target genes, including p21, Bax, Gadd45, and Mdm2, are induced upon p53 activation in CP, providing an *in vivo* assay for p53 transactivation functions (Yin *et al*., 1997; Lu *et al*., 2001; and TVD unpublished). Thus, to test $p53^{QSval}$ function in response to proliferative stress *in vivo*, we generated TgT_{121} mice in which one or both *p53* alleles harbored the $p53^{QSval}$ mutations.

Table 2 LOH in tumors of heterozygous mice

Mouse #	*Age (months)*	*Sex*	*Tumor type*	*LOH*
$Trp53^{+/-}$ mice				
4F2KO	12.8	F	Sarcoma (osteo-)	No
15F2KO	22.8	F	Lymphoma (general)	No
35F2KO	13.9	F	Sarcoma	No
41F2KO	25.5	M	Lymphoma (general)	No
55F2KO	17.3	F	Lymphoma (general)	LOH
21F3KO	12.9	F	Other (carcinoma)	No
104KO	15.0	F	Sarcoma (fibro-)	LOH
129KO	18.2	F	Sarcoma (osteo-)	No
130KO	18.2	F	Sarcoma (osteo-)	No
$Trp53^{+/QSval}$ mice				
56F2QS	19.7	M	Sarcoma (angio-)	LOH
15F3QS	14.7	F	Sarcoma (fibro-)	No
16F3QS	18.1	F	Sarcoma (fibro-)	No
18F3QS	18.3	M	Lymphoma (general)	No
19F3QS	14.7	M	Sarcoma (osteo-)	LOH
31F3QS	15.5	M	Sarcoma (low diff.)	No
1F4QS	16.9	M	Lymphoma (general)	No
9F4QS	21.2	M	Sarcoma (osteo-)	LOH
28F4QS	20.6	M	Lymphoma (general)	No
33F4QS	14.3	F	Lymphoma (thymic)	No
35F4QS	20.1	M	Sarcoma (lipo-)	No
46F4QS	18.6	F	Other (sq. cell carcinoma)	No
66F4QS	17.9	M	Other (carcinoma)	LOH
69F4QS	20.7	M	Lymphoma (general)	No
73F4QS	16.4	F	Lymphoma (general)	No
78F4QS	7.7	F	Lymphoma (thymic)	LOH
80F4QS	16.4	F	Other (anaplastic tumor)	No
85F4QS	6.9	M	Sarcoma (angio-)	No

Analysis of CP apoptosis indicated that the $p53^{QSval}$ allele behaves as a null allele with respect to this function. The apoptosis was high in TgT_{121} tissue, and it was reduced in TgT_{121} $p53^{QSval/QSval}$ tissue to the same extent as in TgT_{121} $p53^{-/-}$ tissue. Furthermore, this decrease in apoptosis resulted in the same acceleration of tumor growth and reduction in survival time as observed by complete inactivation of *p53* (Figure 6), thus establishing the link between p53 transcriptional regulation, apoptosis, and tumor suppression.

The TgT_{121} tumor model is also useful for the examination of tumor progression under selective pressures elicited by p53-dependent apoptosis. In TgT_{121} $p53^{+/-}$ mice from birth to about 6 weeks of age, the CP is dysplastic and consists of both proliferating and apoptosing cells. However, at 6–8 weeks, focal solid tumors arise that exhibit decreased apoptosis as well as angiogenesis (Lu *et al*., 2001). Tumors grow aggressively causing death by an average of 12 weeks (Figure 6a), and 90% undergo selective loss of the wild-type *p53* allele. In the remaining 10% of tumors, p53 is inactive based on the loss of p53-dependent p21 induction (Pan *et al*., 1998; Lu *et al*., 2001).

In each of these assays, $p53^{QSval}$ functioned as a null allele. Survival of TgT_{121} $p53^{+/QSval}$ mice followed the same kinetics as that of TgT_{121} $p53^{+/-}$ mice (Figure 6a). Analysis of tumor morphology during progression showed the same development of focally aggressive tumors within the same timeframe (Figure 7A, f and h).

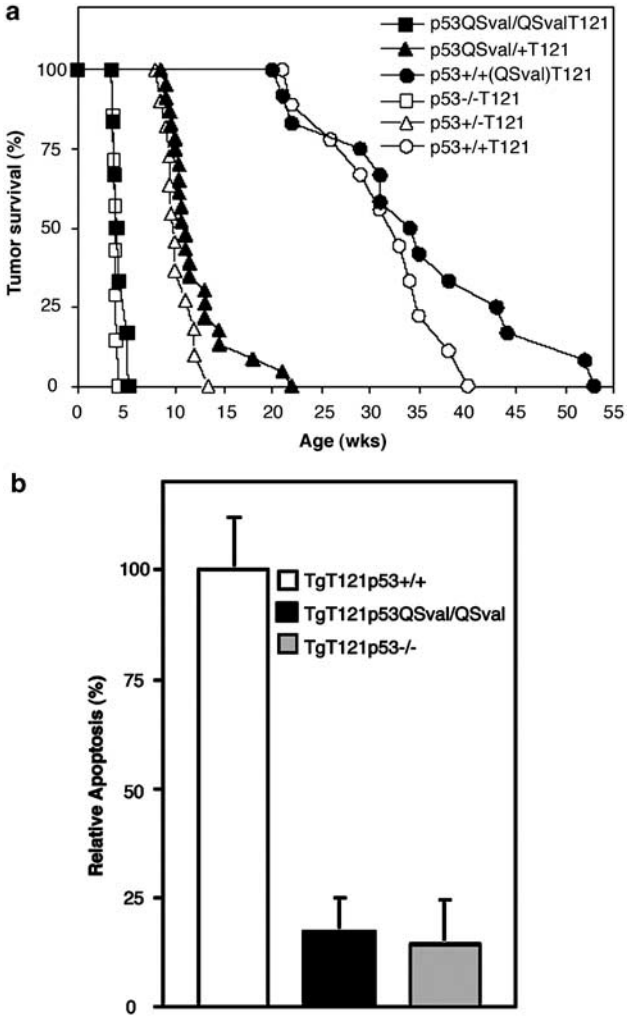

Figure 6 $p53^{QSval}$ and *p53*-null alleles similarly accelerate brain tumor growth and attenuate apoptosis in TgT_{121} mice. (**a**) Tumor survival. TgT_{121} mice with no, one or two $p53^{QSval}$ allele(s) (solid figures) were monitored. Survival times reflect the time of killing dictated by the presence of a bulged cranium. Tumor survival of TgT_{121} mice with no, one, or two *p53*-null allele(s) (empty figures) was plotted for comparison (one mouse per data point). (**b**) Relative apoptosis indexes (AIs) within the choroid plexus of $TgT_{121};p53^{QSval/QSval}$ mice and TgT_{121} littermates. The TgT_{121} value is 100%. AIs were quantified for three mice in each group

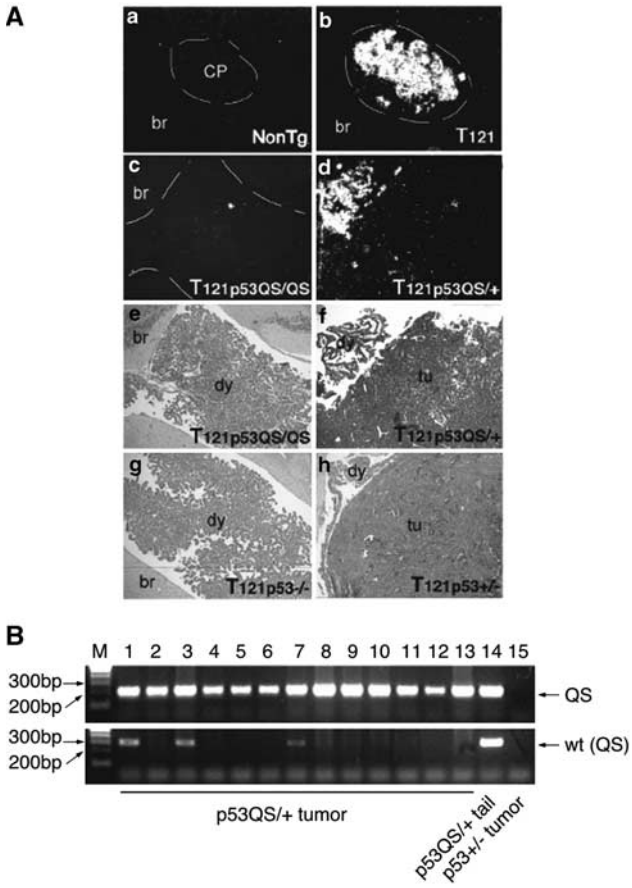

Figure 7 p21 mRNA expression (**A**) and p53 LOH (**B**) in brain tumors of $TgT_{121};p53^{QSval}$ mice (*QSval* labeled as QS). (**A**) Choroid plexus (CP) of normal mice does not express p21 mRNA (**a**), while p21 is induced in TgT_{121} mice (**b**). CP tumors from $TgT_{121};p53^{QSval/QSval}$ (**c**, **e**) and $TgT_{121};p53^{+/QSval}$ (**d**, **f**) terminal mice were examined for p21 expression by *in situ* RNA hybridization (**c**, **d**) and for tumor morphology (**e**, **f**). The CP-containing region is framed with dotted lines. The p21 transcript is undetectable in $TgT_{121};p53^{QSval/QSval}$ (**c**, **e**) and in the solid tumor of $TgT_{121};p53^{+/QSval}$ (**d**, **f**), while it is expressed in adjacent dysplastic CP (**d**, **f**). In comparison, H&E stains of TgT_{121} tumors with $p53^{-/-}$ (**g**) and $p53^{+/-}$ (**h**) genotypes are presented. p21 RNA was previously shown to be absent in $p53^{-/-}$ and p53 LOH tumors in the same manner as shown here for $p53^{QSval}$ (Lu *et al*., 2001). Note the similarities of tumors in heterozygotes and homozygotes of $p53^{QSval}$ and null alleles, respectively. Br, brain; dy, dysplastic CP; tu, solid tumor. (**B**) Genomic DNAs from $TgT_{121};p53^{+/QSval}$ terminal tumors (lanes 1–13) and tail DNA (lane 14) and a $TgT_{121};p53^{+/-}$ terminal tumor (lane 15) were analysed by a semiquantitative allele-specific PCR to detect *QSval* (upper panel) and *wt* (lower panel) *p53* alleles

Moreover, *in situ* hybridization for p21 RNA showed that p53 remained active in dysplastic tissue of $p53^{QSval}$ heterozygotes (Figure 7A, d and f) as in *p53*-null heterozygotes, indicating that p53 was functioning as expected and was not affected by the presence of abundant $p53^{QSval}$ protein. Furthermore, tumors arising from dysplastic tissue within the same section were devoid of p21 RNA, indicative of loss of p53 function (Figure 7A, d and f).

To determine whether the selective pressure for wild-type p53 inactivation had been affected by the presence of a single $p53^{QSval}$ allele, terminal tumors were analysed for LOH using semiquantitative allele-specific PCR analysis. Of 13 tumors analysed, 10 showed clear loss of the wild-type *p53* allele, while all tumors retained the $p53^{QSval}$ allele (Figure 7B). Thus, the high selective pressure for inactivation of the wild-type *p53* allele is unchanged in TgT_{121} $p53^{+/QSval}$ mice compared to TgT_{121} $p53^{+/-}$ mice. Furthermore, the complete retention of the $p53^{QSval}$ allele in progressed tumors confirms that this allele behaves as a null allele. Taken together, in the context of tumor growth and progression initiated by pRb pathway disruption, $p53^{QSval}$ alleles behave as *p53*-null alleles with respect to apoptotic response, selective pressure for wild-type *p53* allele loss, and the morphological and biological characteristics of tumor progression.

Discussion

The studies presented here aimed to determine the role of transcriptional regulation function of p53 in tumor suppression. Mutations in codons 25 and 26 interfere with association of p53 and the basal transcriptional machinery (Lin *et al*., 1994), and should thus produce a severely disabled regulatory protein. Indeed, we showed that when expressed at physiological levels, the mutated p53 protein ($p53^{QSala}$) failed to induce a number of target genes. Similar results were obtained in differentiated ES cells harboring an independently generated $p53^{QS}$ mutant (Chao *et al*., 2000). These observations indicate that a second proposed N-terminal transactivation domain (Venot *et al*., 1999) either does not function at physiologically relevant expression levels, or that it must depend on the N-terminal domain containing residues 25 and 26.

C-terminal acetylation of p53 was initially reported to be required for its binding to p53 response elements (Gu and Roeder, 1997), but recent studies have challenged this concept (Espinosa and Emerson, 2001). Our chromatin immunoprecipitation (ChIP) results additionally confirmed that acetylation of p53 is not necessary for its DNA-binding activity as $p53^{QSala}$, which is not detectably acetylated (Chao *et al*., 2000), binds to native chromatin as strongly as the damage-activated wild-type protein. Moreover, $p53^{QSala}$ binds DNA in the absence of any stress, implying that stress-induced modifications of p53 do not play an essential role in regulating DNA binding. Therefore, p53 activation must be achieved by other mechanisms such as recruitment of coactivators and modification of chromatin structure (Barlev *et al*., 2001) or destruction of the negative regulator, MDM2 (Stommel and Wahl, 2004).

In several systems, the ability of p53 to suppress tumor formation depends on its ability to generate an apoptotic response. Both transcription-dependent and -independent mechanisms have been proposed for p53-induced apoptosis. Recently, a role for p53 in the direct induction of mitochondrial apoptosis, independent of transcriptional regulation has emerged (Dumont *et al*., 2003; Mihara *et al*., 2003; Chipuk *et al*., 2004; Leu *et al*., 2004). It is noteworthy that MEFs obtained from the $p53^{QSval}$ mice we generated did undergo apoptosis when treated with wheat germ agglutinin, which was taken as evidence supporting a cytoplasmic, transcription-independent apoptotic mechanism (Chipuk *et al*., 2004). In addition, interaction between the p53 central DNA-binding domain and Bcl-2 family members, Bak or BclXL (Mihara *et al*., 2003; Leu *et al*., 2004), has also been reported to induce transcription-independent apoptosis. However, our data clearly demonstrate that $p53^{QSval}$ does not induce apoptosis *in vitro* or *in vivo* under a variety of conditions, and is completely unable to suppress tumor formation in spontaneous and oncogene-initiated models. Furthermore, consistent with our data concerning $p53^{QSala}$, Chao *et al*. (2000) also observed defective apoptosis in $p53^{QS}$ thymocytes, indicating that defective apoptosis is most likely a direct consequence of the defective transactivation domain generated by the $p53^{QS}$ mutations. Taken together, these data strongly suggest that p53 elicits apoptotic function through its transcriptional regulation activity. However, we cannot exclude the possibility that inefficient translocation of either $p53^{QSval}$ or $p53^{QSala}$ proteins to the cytoplasm also contributes to reducing the effectiveness of the apoptotic response.

Here, we employed several mouse model systems to measure the tumor suppression function of the $p53^{QSval}$ mutant. One system examines p53 in suppressing spontaneous tumorigenesis; the other determines the role of p53 in suppressing oncogene-initiated tumor formation. In each system, the $p53^{QSval}$ allele behaves like a *p53*-null allele. $p53^{QSval}$ and *p53*-null mice exhibit identical tumor kinetics and tumor spectra. In addition, the presence of the abundant $p53^{QSval}$ protein does not alter the selective pressure for specific loss of the wild-type *p53* allele in heterozygous mice. The most direct interpretation of these data is that $p53^{QSval}$ is inactive with regard to *in vivo* biological functions in mouse tissues at risk for tumor formation.

Given the severe impact of the QS mutations on transactivation, it is not surprising that $p53^{QS}$ proteins with or without the val135 mutation exhibit no biological differences. In support of this interpretation, the $p53^{QS}$ mutation introduced into ES cells independently by Chao *et al*. (2000) using a different genomic targeting construct showed identical biologic properties as those of $p53^{QSval}$. Furthermore, although several reports showed that $p53^{val135}$ expressed at high levels in transgenic mice elicited tumor predisposition (Lavigueur *et al*., 1989; Harvey *et al*., 1995), the tumor spectrum differed from that of the $p53^{QSval}$ mice described in this work. Intriguingly, another group reported no adverse effect when the same mutant was expressed at high levels in several tissues (Lozano and Levine, 1991). Studies with human p53 showed that the val135 mutation still allows p53 to induce apoptosis in E1A-Ras-transformed MEFs, while the triple mutation L22QW23Sval135 completely prevents apoptosis (Sabbatini *et al*., 1995). This supports our interpretation that the QS mutations are so severe with regard to compromising the transcriptional function of p53 that the val135 mutation does not exert additional negative effects. Finally, although the val135 mutation was reported to act in a dominant negative fashion to accelerate tumor development in mice carrying wild-type *p53* alleles (Harvey *et al*., 1995), we show here that $p53^{QSval}$ does not act in this manner *in vivo*, nor does it interfere with wild-type p53 in a skin tumorigenesis assay (Weinberg, Nistér, Wahl *et al*., in preparation).

Taken together, the compelling tumorigenicity and functional data suggest that the severe defect in transcriptional regulation caused by the QS mutations accounts for the loss of tumor suppression in multiple mouse tissues. We did not find any evidence that binding of transcriptionally inactive p53 to its responsive promoters could contribute to tumor suppression. Our data indicate that other proposed transcription-independent functions of p53, such as direct interaction with DNA repair proteins, either are not important for tumor

suppression, or must depend on the N-terminal transactivation domain and thus play a role in concert with or downstream of transcriptional regulation. However, our data do not exclude the possibility that additional transcription-independent functions mediated by the central DNA-binding domain may contribute to the efficiency of tumor suppression in some tissues or under specific conditions not examined here. On the other hand, the extremely high incidence of DNA-binding domain mutants in human cancers, combined with our analyses, lead us to propose that the transcriptional regulatory function of p53 is paramount for tumor suppression.

Materials and methods

Mice

$p53^{QSval}$ mice (Jimenez *et al*., 2000) were bred to $\geqslant$75% C57Bl/6 background before initiation of the tumorigenesis studies described here. The *p53*-null mice (Donehower *et al*., 1992) had a similar C57Bl/6 background (close to 100% C57Bl/6) at the time of these studies. $p53^{+/QSval}$ mice were intercrossed for several generations as were $p53^{+/-}$ mice. Mice were killed when visible tumors appeared or upon symptoms of disease. Most mice were followed for up to 15 months, while a subgroup was followed further to enable tumor detection in heterozygotes.

To generate TgT_{121};$p53^{+/QSval}$ and TgT_{121};$p53^{QSval/QSval}$ mice for the analysis of brain tumor formation, $p53^{QSval/QSval}$ mice were crossed to TgT_{121} mice for one or two generations, respectively. Generation, screening, and characterization of TgT_{121} (B6D2) mice were described previously (Symonds *et al*., 1994). Mice utilized for apoptosis assays were killed at 3–4 weeks prior to tumor progression. Mice for survival analysis were killed when the presence of brain tumors was apparent by cranial bulging.

Mouse genotyping and detection of allele loss in tumors

Allele analysis was carried out by PCR (see Supplementary information).

Analysis of mice

All mice were autopsied. Heart, lungs, thymus, spleen, lymph nodes, liver, brain, and digestive and urogenital organs were macroscopically examined for spontaneous tumor formation. Some mice presented with paralysis of hind legs, in which cases the vertebral column and spinal cord were investigated. Organs with macroscopically visible alterations were sampled, and samples fixed in 4% paraformaldehyde, embedded in paraffin, sectioned and stained with H&E for histopathological evaluation. All sections were evaluated by the same investigator. Tumors were categorized as lymphoma, sarcoma, and 'other' to enable statistical comparison of tumor types between genotypes. Lymphomas were categorized as thymic lymphoma when only localized in thymus and other parts of the thoracic cavity and as 'generalized' when also involving other organs or found only in extrathoracic location(s). Sarcomas were also subclassified where possible. Samples from tumor and normal tissue were frozen in liquid nitrogen and kept at –70°C for genotype analysis. Brains from mice with TgT_{121} genotypes were analysed after fixation and paraffin embedding.

Statistical methods

Survival curves were calculated with the Kaplan–Meier method. Cause-specific survival was used; mice affected by causes other than tumors and mice alive at the end of follow-up were censored. Differences in survival were analysed with the log rank test. The median survival from different tumors was used as a surrogate marker for tumor latency. Difference in proportions of tumors between groups were analysed with χ^2 or Fisher exact test in cases where less than five mice comprised a group. All analyses were performed with the Statistica Software 6.0.

In situ *assays*

Paraffin-embedded brain sections were analysed for apoptosis using the Apoptag kit (Intergen, NY, USA) and results were quantified as previously described (Symonds *et al*., 1994; Yin *et al*., 1997). *In situ* RNA hybridization was carried out as previously described (Pan *et al*., 1998).

Immunoprecipitation, ChIP, and real-time quantitative PCR

ChIP assay was performed as described (Kaeser and Iggo, 2002) with modifications described in detail in Supplementary Information.

Generation of stable cell lines expressing $p53^{QSala}$*, cell culture, apoptosis assay, and xenograft assay in athymic nude mice*

The open reading frame of $p53^{QSala}$ was cloned into lentiviral expression vector pLenti6/V5-DEST (Invitrogen) using the Invitrogen Gateway System. A *Cla*I–*Bam*HI fragment containing the CMV promoter was removed and replaced with a *Pac*I–*Bam*HI fragment containing the mouse ubiquitin C promoter from plasmid c-FUW (provided by D Baltimore) following Klenow fill-in. Lentiviruses were prepared from the resulting construct, pLentiQS-Ubc, and used to infect early passage $p53^{-/-}$ MEF cells following the manufacturer's protocol. Stable clones expressing $p53^{QSala}$ at levels similar to the endogenous $p53^{QSval}$ were picked and expanded after selection in 3 μg/ml blasticidin. MEFs were maintained in DMEM plus 15% FBS, 100 μM β-mercaptoethanol, 2 mM L-glutamine, antibiotics, and for $p53^{QSala}$ clones 3 μg/ml blasticidin.

Preparation of retroviruses expressing E1A-12S or Ha-ras, retrovirus infection, and xenograft assays in athymic nude mice were as described Jimenez *et al*. (2000). E1A- and Ras-expressing MEFs were treated with 0.2 μg/ml adriamycin or 2.5 μM etoposide to induce apoptosis. After 24h, floating and adherent cells were collected and stained with annexin V-FITC. Apoptosis was scored by flow cytometry.

Note Added in Proof

We recently obtained new stable cell lines expressing $p53^{QSala}$, and found that several grew more slowly than either $p53^{QSval}$ MEFs, or $p53^{QSala}$ subclone 5 used in the studies in this paper. The $p53^{QSala}$ expression in the new clones was high, and heterogeneous, suggesting that compromised growth may result from very high expression of $p53^{QSala}$. It is possible that $p53^{QSala}$ does retain some transcriptional activity manifested when it is expressed at high levels, or that excessive levels of a transcriptionally inactive protein that binds stably to numerous promoters and cannot be removed by proteolysis can adversely affect cell growth.

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References

Appella E and Anderson CW. (2001). *Eur. J. Biochem.*, **268,** 2764–2772.
Barlev NA, Liu L, Chehab NH, Mansfield K, Harris KG, Halazonetis TD and Berger SL. (2001). *Mol. Cell*, **8,** 1243–1254.
Brooks CL and Gu W. (2003). *Curr. Opin. Cell Biol.*, **15,** 164–171.
Caelles C, Helmberg A and Karin M. (1994). *Nature*, **370,** 220–223.
Candau R, Scolnick DM, Darpino P, Ying CY, Halazonetis TD and Berger SL. (1997). *Oncogene*, **15,** 807–816.
Chao C, Saito S, Kang J, Anderson CW, Appella E and Xu Y. (2000). *EMBO J.*, **19,** 4967–4975.
Chipuk JE, Kuwana T, Bouchier-Hayes L, Droin NM, Newmeyer DD, Schuler M and Green DR. (2004). *Science*, **303,** 1010–1014.
Donehower LA, Harvey M, Slagle BL, McArthur MJ, Montgomery Jr CA, Butel JS and Bradley A. (1992). *Nature*, **356,** 215–221.
Dumont P, Leu JI, Della Pietra III AC, George DL and Murphy M. (2003). *Nat. Genet.*, **33,** 357–365.
Espinosa JM and Emerson BM. (2001). *Mol. Cell*, **8,** 57–69.
Gu W and Roeder RG. (1997). *Cell*, **90,** 595–606.
Harvey M, Vogel H, Morris D, Bradley A, Bernstein A and Donehower LA. (1995). *Nat. Genet.*, **9,** 305–311.
Haupt Y, Rowan S, Shaulian E, Kazaz A, Vousden K and Oren M. (1997). *Leukemia*, **11** (Suppl. 3)**,** 337–339.
Haupt Y, Rowan S, Shaulian E, Vousden KH and Oren M. (1995). *Genes Dev.*, **9,** 2170–2183.
Hollstein M, Sidransky D, Vogelstein B and Harris CC. (1991). *Science*, **253,** 49–53.
Jacks T, Remington L, Williams BO, Schmitt EM, Halachmi S, Bronson RT and Weinberg RA. (1994). *Curr. Biol.*, **4,** 1–7.
Jimenez GS, Nister M, Stommel JM, Beeche M, Barcarse EA, Zhang XQ, O'Gorman S and Wahl GM. (2000). *Nat. Genet.*, **26,** 37–43.
Kaeser MD and Iggo RD. (2002). *Proc. Natl. Acad. Sci. USA*, **99,** 95–100.
Lavigueur A, Maltby V, Mock D, Rossant J, Pawson T and Bernstein A. (1989). *Mol. Cell. Biol.*, **9,** 3982–3991.
Leu JI, Dumont P, Hafey M, Murphy ME and George DL. (2004). *Nat. Cell Biol.*, **6,** 443–450.
Levine AJ, Momand J and Finlay CA. (1991). *Nature*, **351,** 453–456.
Lin J, Chen J, Elenbaas B and Levine AJ. (1994). *Genes Dev.*, **8,** 1235–1246.
Liu G, Parant JM, Lang G, Chau P, Chavez-Reyes A, El-Naggar AK, Multani A, Chang S and Lozano G. (2004). *Nat. Genet.*, **36,** 63–68.
Lozano G and Levine AJ. (1991). *Mol. Carcinog.*, **4,** 3–9.
Lu X, Magrane G, Yin C, Louis DN, Gray J and Van Dyke T. (2001). *Mol. Cell. Biol.*, **21,** 6017–6030.
Marchenko ND, Zaika A and Moll UM. (2000). *J. Biol. Chem.*, **275,** 16202–16212.
Martinez J, Georgoff I and Levine AJ. (1991). *Genes Dev.*, **5,** 151–159.
Michael D and Oren M. (2003). *Semin Cancer Biol.*, **13,** 49–58.
Michalovitz D, Halevy O and Oren M. (1990). *Cell*, **62,** 671–680.
Mihara M, Erster S, Zaika A, Petrenko O, Chittenden T, Pancoska P and Moll UM. (2003). *Mol. Cell*, **11,** 577–590.
Milner J and Medcalf EA. (1990). *J. Mol. Biol.*, **216,** 481–484.
Mummenbrauer T, Janus F, Muller B, Wiesmuller L, Deppert W and Grosse F. (1996). *Cell*, **85,** 1089–1099.
Nigro JM, Baker SJ, Preisinger AC, Jessup JM, Hostetter R, Cleary K, Bigner SH, Davidson N, Baylin S, Devilee P, Glover T, Collins FS, Weslon A, Modali R, Harris CC and Vogelstein B. (1989). *Nature*, **342,** 705–708.
Offer H, Milyavsky M, Erez N, Matas D, Zurer I, Harris CC and Rotter V. (2001). *Oncogene*, **20,** 581–589.
Pan H, Yin C, Dyson NJ, Harlow E, Yamasaki L and Van Dyke T. (1998). *Mol. Cell*, **2,** 283–292.
Prives C. (1994). *Cell*, **78,** 543–546.
Rubbi C and Milner J. (2003). *EMBO J.*, **22,** 975–986.
Sabbatini P, Lin J, Levine AJ and White E. (1995). *Genes Dev.*, **9,** 2184–2192.
Schmitt CA, Fridman JS, Yang M, Baranov E, Hoffman RM and Lowe SW. (2002). *Cancer Cell*, **1,** 289–298.
Stommel JM and Wahl GM. (2004). *EMBO J.*, **23,** 1547–1556.
Sturzbecher HW, Donzelmann B, Henning W, Knippschild U and Buchhop S. (1996). *EMBO J.*, **15,** 1992–2002.
Symonds H, Krall L, Remington L, Saenz-Robles M, Lowe S, Jacks T and Van Dyke T. (1994). *Cell*, **78,** 703–711.
Tyner SD, Venkatachalam S, Choi J, Jones S, Ghebranious N, Igelmann H, Lu X, Soron G, Cooper B, Brayton C, Hee Park S, Thompson T, Karsenty G, Bradley A and Donehower LA. (2002). *Nature*, **415,** 45–53.
Venkatachalam S, Shi YP, Jones SN, Vogel H, Bradley A, Pinkel D and Donehower LA. (1998). *EMBO J.*, **17,** 4657–4667.
Venkatachalam S, Tyner SD, Pickering CR, Boley S, Recio L, French JE and Donehower LA. (2001). *Toxicol. Pathol.*, **29,** 147–154.
Venot C, Maratrat M, Sierra V, Conseiller E and Debussche L. (1999). *Oncogene*, **18,** 2405–2410.
Vogelstein B and Kinzler KW. (1992). *Cell*, **70,** 523–526.
Vousden KH. (2000). *Cell*, **103,** 691–694.
Vousden KH and Lu X. (2002). *Nat. Rev. Cancer*, **2,** 594–604.
Wahl GM and Carr AM. (2001). *Nat. Cell Biol.*, **3,** E277–E286.
Yin C, Knudson CM, Korsmeyer SJ and Van Dyke T. (1997). *Nature*, **385,** 637–640.
Zhou J, Ahn J, Wilson SH and Prives C. (2001). *EMBO J.*, **20,** 914–923.
Zhu J, Jiang J, Zhou W, Zhu K and Chen X. (1999). *Oncogene*, **18,** 2149–2155.

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