Cancer Research

Spontaneous Tumorigenesis in Mice Overexpressing the p53-Negative Regulator Mdm4

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Abstract

High levels of the critical p53 inhibitor Mdm4 is common in tumors that retain a wild-type *p53* allele, suggesting that Mdm4 overexpression is an important mechanism for p53 inactivation during tumorigenesis. To test this hypothesis *in vivo*, we generated transgenic mice with widespread expression of Mdm4. Two independent lines of transgenic mice, $Mdm4^{Tg1}$ and $Mdm4^{Tg15}$, developed spontaneous tumors, the most prevalent of which were sarcomas. To determine whether overexpression of Mdm4 also cooperated with *p53* heterozygosity to induce tumorigenesis, we generated $Mdm4^{Tg1}$ $p53^{+/-}$ mice. These mice had significantly accelerated tumorigenesis and a distinct tumor spectrum with more carcinomas and significantly fewer lymphomas than $p53^{+/-}$ or $Mdm4^{Tg1}$ mice. Importantly, the remaining wild-type *p53* allele was retained in most $Mdm4^{Tg1}$ $p53^{+/-}$ tumors. Mdm4 is thus a bona fide oncogene *in vivo* and cooperates with *p53* heterozygosity to drive tumorigenesis. These *Mdm4* mice will be invaluable for *in vivo* drug studies of Mdm4 inhibitors. *Cancer Res; 70(18); 7148–54. ©2010 AACR.*

Introduction

Inactivation of the p53 tumor suppressor is a critical step in tumorigenesis (1). *p53* is mutated or deleted in many human cancers. Other tumors with wild-type p53 have defects in critical regulators of p53, such as $p14^{ARF}$ and Mdm2 (2). Mdm2 inhibits p53 transcriptional activity and mediates the degradation of p53 through its E3 ubiquitin ligase activity (3–5). Amplification of the *MDM2* gene occurs in 30% to 40% of human sarcomas and leukemias, many of which retain wild-type *p53* (6–8). Also, high levels of MDM2 are found in many other tumors (9, 10) and are associated with poor prognosis in patients with non–Hodgkin's lymphoma (11). In mice, overexpression of Mdm2 induces tumorigenesis in a wild-type *p53* background (12). These data show that *Mdm2* is an oncogene *in vivo*.

Another potential mechanism for disrupting the p53 pathway in tumorigenesis is through Mdm4, an Mdm2 homologue that also inhibits p53 function (13, 14). Mouse embryos lacking *Mdm4* die during embryogenesis; however, this phenotype is completely rescued by loss of *p53* (14–16). These data show that Mdm4 is a negative regulator of p53 that is not redundant with Mdm2. Overexpression of Mdm4 with $HRas^{v12}$ transforms mouse embryonic fibroblasts (MEF), suggesting a role in transformation (17). Additionally, MDM4 is highly expressed in a significant percentage of human tumors, including 65% of retinoblastomas (18), 39% of head and neck squamous carcinomas (10), 19% of breast cancers, 19% of colon cancers, 18% of lung cancers (17), and 80% of adult pre-B lymphoblastic leukemia (19). Most retinoblastomas and head and neck squamous carcinomas have wild-type *p53* (10, 18). These studies strengthen the argument that *MDM4* is an oncogene.

Mdm4 also interacts with Mdm2 through its RING finger (20), which leads to its ubiquitination by Mdm2 and subsequent degradation by the 26S proteasome (21–23). DNA damage induces Mdm4 phosphorylation and subsequent ubiquitination and degradation, which is required for the p53-mediated DNA damage response (24–28). However, the role of Mdm4 overexpression in the p53-mediated DNA damage response *in vivo* is unclear.

To investigate the effects of Mdm4 overexpression *in vivo*, we generated transgenic *Mdm4* mice through two strategies. Because previous studies show that high levels of Mdm2 cause embryonic lethality in mice (12), we reasoned that high levels of Mdm4 might also lead to developmental phenotypes. We therefore created a conditional transgenic mouse ($Mdm4^{Tg}$) in which the *Mdm4* transgene is expressed only on deletion of a floxed enhanced green fluorescent protein (EGFP) cassette ($Mdm4^{Tg1}$). $Mdm4^{Tg1}$ mice were viable and developed spontaneous tumors. Another transgenic line, $Mdm4^{Tg15}$, constitutively expressing Mdm4 also showed a cancer phenotype. Lastly, $Mdm4^{Tg1}$ $p53^{+/-}$ mice showed significantly accelerated tumorigenesis with

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retention of wild-type *p53* in most tumors. Mdm4 overexpression also contributed to reduced p53 stability in response to stress. These transgenic *Mdm4* mouse models show a direct role of Mdm4 overexpression in tumorigenesis *in vivo*, and serve as important models for drug screening and cancer therapy.

Materials and Methods

Generation of transgenic Mdm4 mice

Transgenic *Mdm4* mice were generated by pronuclear injection at The University of Texas M.D. Anderson Cancer Center Genetically Engineered Mouse Facility. The transgene was identified by PCR using the following primers: ALF, AGGGCGGGGTTCGGCTTCTGG, and E4re, TCCCAAAAGATCTCCACCACAGTA. To delete *EGFP*, $Mdm4^{Tg}$ mice were mated with *Zp3-Cre* mice, and then with C57Bl/6J mice to generate $Mdm4^{Tg1}$mice.

Western blot analysis

Protein lysates prepared from HeLa and HepG2 cells, MEFs, tissues, and tumors were used for Western blot analyses. HeLa and HepG2 cells were originally obtained from the American Type Culture Collection (ATCC) in 2005, and aliquots were subsequently frozen in liquid nitrogen until time of use. The cells were cultured in Eagle's minimum essential medium supplemented with 10% fetal bovine serum (FBS) as per ATCC recommendations. HeLa and HepG2 cells were authenticated by G-banded karyotyping analysis on July 7, 2010, by the M.D. Anderson Cytogenetics Core Facility. Early-passage MEFs generated from 13.5 days postcoitum embryos and cultured in DMEM supplemented with 10% FBS were used for the analysis. In radiation experiments, 6- to 8-week-old wild-type and transgenic mice were irradiated at 6 Gy and then sacrificed at different time points. Antibodies used for Western blots were p53 (CM5, Novacastra); p21(BD Biosciences); cleaved-caspase 3 (Cell Signaling); Mdm4 antibody (MX82); actin (Santa Cruz Biotechnology, Inc.); vinculin, β-actin, and tubulin (Sigma); and Mdm2 (2A10; Calbiochem).

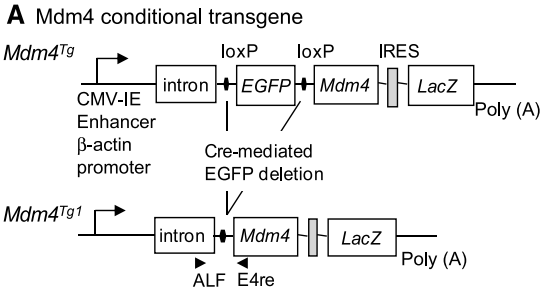

Figure 1. Generation of Mdm4 transgenic mice. A, the chicken *β-actin* promoter and intron with a CMV IE enhancer drive expression of the *Mdm4* cDNA upon deletion of *EGFP*, which is flanked by *loxP* sites. An IRES allows *LacZ* expression. A rabbit β-globin polyadenylation signal [poly(A)] was placed at the 3′ end. ALF and E4 mark the location of primers for genotyping. B, a constitutive *Mdm4* transgene driven by the chicken β-actin promoter and CMV IE enhancer was used to generate $Mdm4^{Tg6}$ and $Mdm4^{Tg15}$ lines.

Table 1. *Mdm4* expression levels

Tissues	$Mdm4^{Tg1}$	$Mdm4^{Tg6}$	$Mdm4^{Tg15}$
Spleen	1.7 ± 1.0	2.9 ± 0.6	6.7 ± 2.4
Thymus	1.5 ± 0.4	2.6 ± 0.6	3.1 ± 1.1
Muscle	6.5 ± 0.1	56.3 ± 12.8	23.1 ± 7.4
Liver	1.1 ± 0.2	1.2 ± 0.1	8.7 ± 2.9
Intestine	2.1 ± 0.1	8.4 ± 1.7	16.8 ± 4.2
Kidney	0.8 ± 0.1	1.2 ± 0.1	3.3 ± 0.6
Heart	6.5 ± 0.1	29.6 ± 1.5	18.0 ± 4.4
Lung	0.8 ± 0.1	2.1 ± 0.1	10.0 ± 2.3

NOTE: The expression levels were compared with tissues from wild-type littermates as fold change determined by RT-qPCR.

Real-time quantitative PCR

Splenocytes were isolated by mashing spleens between the rough part of superfrost slides (Fisher Scientific) and suspended in warm RPMI medium (10% FBS). The cell mixture was then passed through a nylon fiber–filled mini-column (Wako). The cells were spun down and suspended in 5 mL RBC lysis buffer (eBioscience) for 5 minutes. Total RNA was isolated from splenocytes, tissues, and MEFs using Trizol reagent (Invitrogen), and then treated with DNase. cDNAs were made using a first-strand reverse transcriptase kit (GE Healthcare). *p21, Mdm2, Puma*, and *Gapdh* primers were previously described (29). *Mdm4* primers for real-time quantitative PCR (RT-qPCR) were GGAAAAGCCCAGGTTTGACC and GCCAAATCCAAAAATCCCACT.

Loss of heterozygosity of p53 allele assay

Tumor DNA was digested with *Eco*RI and *Stu*I following a published protocol (30).

Statistical analysis

Student's *t* test and Kaplan-Meier survival analysis were performed by using Prism 4 software (GraphPad Software). Differences were considered significant at $P < 0.05$.

Results

Transgenic mice express varying levels of Mdm4

To prevent the potential toxic effects of Mdm4 overexpression on embryonic development, we first generated

a conditional mouse model for Mdm4 overexpression. The *Mdm4* transgene is driven by the cytomegalovirus (CMV) immediate-early (IE) enhancer, and the chicken *β-actin* promoter with an intron that drives widespread expression of genes in a number of transgenic models (31). The construct also contained *EGFP* flanked by *loxP* sites followed by the *Mdm4* cDNA, an internal ribosomal entry site (IRES), the *lacZ* gene, and an SV40 poly(A) sequence (Fig. 1). Using this conditional strategy, the entire transgene will be transcribed, and *EGFP* and *lacZ* will be translated into protein. Deletion of *EGFP* allows translation of *Mdm4* and *lacZ*. Transgenic mice were generated and crossed to C57Bl/6J mice. Embryos were screened by whole-mount X-gal staining at embryonic day 13.5. We identified one transgenic line with intense and extensive X-gal staining (referred to as $Mdm4^{Tg}$; Supplementary Fig. S1A). These mice were subsequently

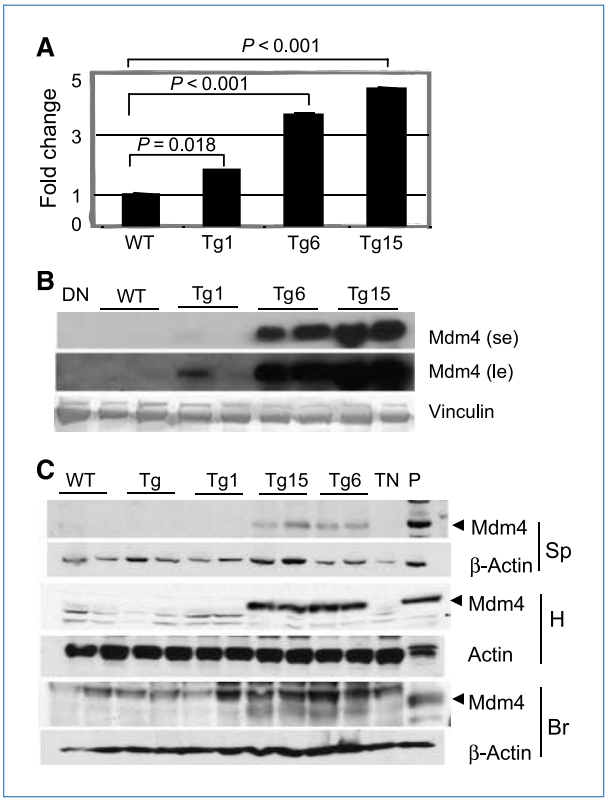

Figure 2. Mdm4 overexpression in mouse tissues of different transgenic lines. A, *Mdm4* mRNA levels in MEFs from $Mdm4^{Tg1}$, $Mdm4^{Tg6}$, and $Mdm4^{Tg15}$ lines were determined by RT-qPCR and plotted as fold change compared to *Gapdh* with wild-type (WT) control set to 1. Three different MEF lines were analyzed for each genotype (*P* values were calculated by Student's *t* test). B, Mdm4 protein levels in MEFs from different lines were assayed by Western blotting. se, short exposure; le, long exposure. C, Western blots from various tissues of 2-month-old transgenic $Mdm4^{Tg}$, $Mdm4^{Tg1}$, $Mdm4^{Tg6}$, and $Mdm4^{Tg15}$ mice using an Mdm4 antibody. β-Actin was used to determine equal loading of the same blot except for heart (H) as that blot was run longer to eliminate overlap with a nonspecific band. Sp, spleen; Br, brain; DN, protein lysate from double-null ($Mdm4^{-/-}$ $p53^{-/-}$) cells; TN, protein lysate from triple-null ($Mdm2^{-/-}$ $Mdm4^{-/-}$ $p53^{-/-}$) cells; P, protein lysate from $Mdm4^{Tg6}$ MEFs as positive control.

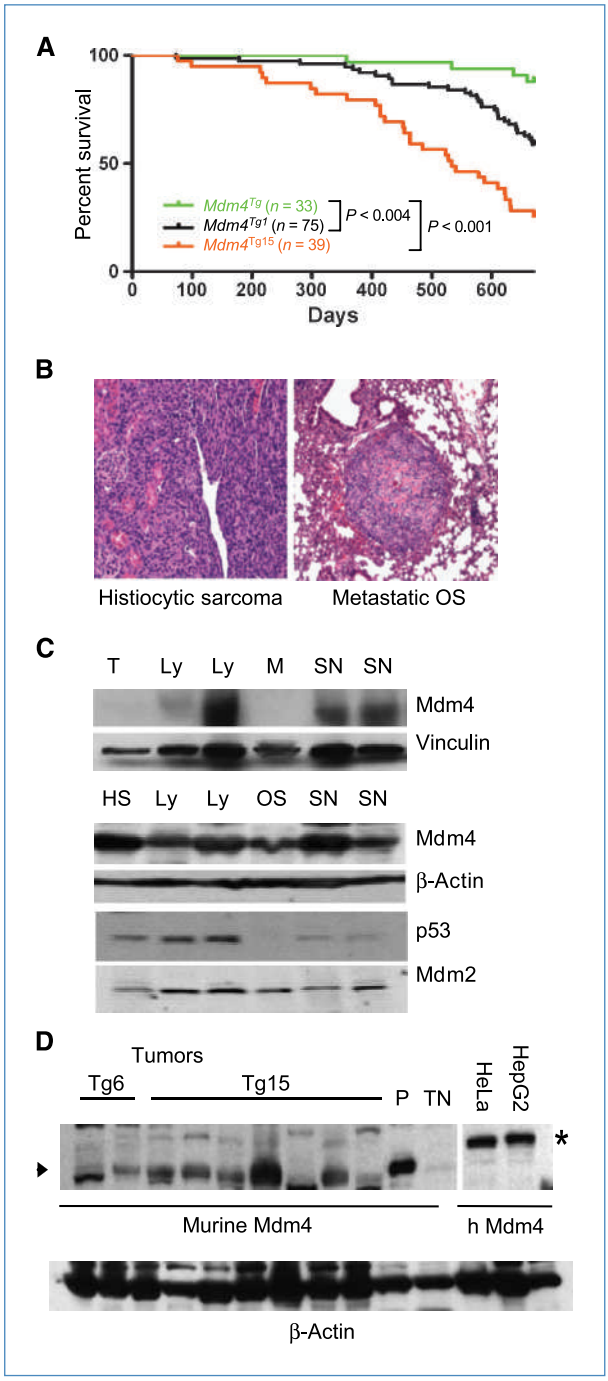

Figure 3. Spontaneous tumorigenesis in $Mdm4^{Tg1}$ and $Mdm4^{Tg15}$ mice. A, Kaplan-Meier survival curves for $Mdm4^{Tg}$, $Mdm4^{Tg1}$, and $Mdm4^{Tg15}$ mice. B, hematoxylin and eosin staining of spontaneous tumors from $Mdm4^{Tg1}$ mice: histiocytic sarcoma (20×) and a metastatic osteosarcoma (OS) to the lung (10×). C, Western blots from $Mdm4^{Tg1}$ tumors and normal tissues using Mdm4, p53, and Mdm2 antibodies. β-Actin and vinculin antibodies were used as a control for loading. T, thymus; M, muscle; HS, histiocytic sarcoma; Ly, lymphoma; OS, osteosarcoma; SN, sarcoma, not otherwise specified. D, Western blot analysis of $Mdm4^{Tg6}$ and $Mdm4^{Tg15}$ tumors, HepG2, and HeLa human tumor cell lines for comparison. *, human (h) MDM4; arrowhead, murine Mdm4 proteins. TN, protein lysate from triple-null ($Mdm2^{-/-}$ $Mdm4^{-/-}$ $p53^{-/-}$) cells; P, protein lysate from $Mdm4^{Tg6}$ MEFs as positive control.

Table 2. Tumor spectra in *Mdm4* transgenic mice

Tumor types	*Mdm4*^{Tg1} (*n* = 24)	*p53*$^{+/-}$ (*n* = 44)	*Mdm4*^{Tg1}*p53*$^{+/-}$ (*n* = 44)	*Mdm4*^{Tg15} (*n* = 13)
Sarcoma	9 (37%)	30 (68%)	28 (64%)	4 (31%)
Hemangiosarcoma	4	3	1	0
Osteosarcoma	1*	15	18	0
Sarcoma, NOS†	4	12	7	3‡
Malignant peripheral Nerve sheath tumor	0	0	1	1
Neurofibrosarcoma			1	
Carcinoma	3 (13%)	2 (5%)	5 (11%)	1 (7%)
Adenocarcinoma	2	1	4	0
Spindle cell carcinoma	0	1	0	0
Squamous cell carcinoma	1	0	1	0
Hepatocellular carcinoma	0	0	0	1
Lymphoma	8 (33%)	10 (23%)	5 (11%)	4 (31%)
Histiocytic sarcoma§	4 (17%)‖	2 (5%)	6 (14%)	4 (31%)‖

*This osteosarcoma had metastasis.
†Not otherwise specified.
‡One of three sarcomas NOS had metastasis.
§Macrophage origin.
‖Two of four histiocytic Sarcomas had metastasis.

crossed with *Zp3-Cre* mice (also in a C57Bl/6J background) to delete the *EGFP* cassette in female germ cells (32), and crossed once again to C57Bl/6J mice to generate *Mdm4*^{Tg1} mice that were >87% C57Bl/6J. Detailed analysis of *Mdm4*^{Tg1} mice showed positive X-gal staining in the retina on postnatal day 2, and in the heart, kidney, and uterus at 2 months of age (Supplementary Fig. S1B and C), suggesting that overexpression of the transgene continues throughout the mouse's life span. In 2-month-old mice, expression of *Mdm4* was also determined by RT-qPCR and Western blotting in multiple organs. The heart, muscle, and intestine showed significantly higher expression of Mdm4 than wild-type controls (Table 1; Supplementary Fig. S1D).

Because Mdm4 transgenic mice did not have obvious developmental defects, we subsequently used a constitutive strategy to drive Mdm4 overexpression (Fig. 1B). We obtained two additional transgenic mouse lines, *Mdm4*^{Tg6} and *Mdm4*^{Tg15}. RT-qPCR showed that both lines also had widespread expression of *Mdm4* in MEFs, spleen, thymus, lung, intestine, heart, muscle, liver (*Mdm4*^{Tg15} only), and kidney (*Mdm4*^{Tg15} only; Fig. 2A; Table 1). Western blot analysis of MEFs, spleen, heart, and brain tissues also showed high levels of Mdm4 protein (Fig. 2B and C). Mdm4 protein was not detectable in wild-type spleen and heart tissues, and is barely visible in the brain (data not shown). In general, the expression of Mdm4 was higher in *Mdm4*^{Tg6} and *Mdm4*^{Tg15} MEFs and tissues than in *Mdm4*^{Tg1} samples (Fig. 2). Western blots for Mdm2 and p53 were also performed and show undetectable levels of these proteins. All three transgenic lines contain a single copy of the transgene (Supplementary Fig. S1E).

Spontaneous tumorigenesis in Mdm4 transgenic mice

We monitored spontaneous tumorigenesis in *Mdm4*^{Tg1} mice and observed that these mice developed tumors earlier than control *Mdm4*^{Tg} mice with the *EGFP* transgene, which did not express *Mdm4* but had the same integration site (Fig. 3). *Mdm4*^{Tg1} mice developed a variety of tumors and died significantly faster than control *Mdm4*^{Tg} mice ($P < 0.004$; Fig. 3A). Twenty-six percent of the *Mdm4*^{Tg1} mice (20 of 75) had tumors during the 18 months observation period, and 20% of these had more than one type of tumor. Tumor types observed were sarcomas (38%), lymphomas (33%), histiocytic sarcoma of macrophage origin (17%; Fig. 3B), and carcinomas (13%; Table 2). One osteosarcoma metastasized to the lung (Table 2; Fig. 3B; Supplementary Table S1). Mdm4 levels were higher in tumors compared with normal tissues, for example, lymphoma versus normal thymus and sarcoma versus normal muscle (Fig. 3C; Supplementary Fig. S2). Six tumors that were well separated from the surrounding normal tissues had high levels of Mdm4 and Mdm2 as shown by Western blot analyses (Fig. 3C). p53 was also high in five of six tumors (Fig. 3C). The sequences of p53 cDNAs cloned from these six tumors were all wild type for *p53*. Additional studies of a second transgenic mouse line, *Mdm4*^{Tg15} (75% C57Bl/6J), also showed that these mice had shortened life spans and developed spontaneous tumors ($P < 0.0001$ compared with *Mdm4*^{Tg} mice). *Mdm4*^{Tg15} mice developed 31% histiocytic sarcomas (including two metastases), 31% sarcomas (one tumor had metastasis to the spleen), 31% lymphomas, and 7% carcinomas. Four of 11 mice developed more than one type of tumor (Supplementary Table S1). Preliminary data from

Mdm4^{Tg6} mice (3 of 29) indicated development of lymphomas and adenomas. Mdm4 protein levels were high in five of seven *Mdm4*^{Tg15} tumors and both *Mdm4*^{Tg6} tumors as shown by Western blot analysis (Fig. 3D). For comparison, protein lysates from HeLa and HepG2 human tumor cell lines also showed high levels of Mdm4. These data indicate that overexpression of Mdm4 contributed to a tumor phenotype in mice.

Tumors develop in *p53* heterozygous mice at a modest rate resembling human cases of Li-Fraumeni syndrome (30, 33). In approximately half of these tumors, loss of one *p53* allele is associated with loss of the second *p53* allele. Tumors retaining wild-type p53 likely acquire other changes to inactivate the pathway (30, 34). To test the hypothesis that Mdm4 overexpression cooperates with *p53* heterozygosity to promote tumorigenesis, we crossed *Mdm4*^{Tg1} mice with *p53* heterozygous mice (also in a C57Bl/6J background) and monitored spontaneous tumorigenesis. The median survival for the *Mdm4*^{Tg1} *p53*$^{+/-}$ mice was 388 days, which was significantly shorter than *Mdm4*^{Tg1} littermates, in which <50% of the mice died during 18 months of observation, and *p53*$^{+/-}$ mice [578 days (P < 0.0001); Fig. 4A]. *Mdm4*^{Tg1} *p53*$^{+/-}$ mice, however, lived longer than *p53*$^{-/-}$ mice (median survival 160 days; Fig. 4A). Additionally, 11% of the tumors (5 of 44) in *Mdm4*^{Tg1} *p53*$^{+/-}$ mice were carcinomas, which was higher than the percentage of carcinomas in p53$^{+/-}$ littermates (5%). Conversely, the percentage of double-mutant mice with lymphomas (11%) was significantly lower than that of the *p53*$^{+/-}$ mice (23%; P < 0.02; Table 2). Interestingly, one *Mdm4*^{Tg1} *p53*$^{+/-}$ mouse had a neurofibrosarcoma, which has not been reported in *p53*$^{+/-}$ mice (Table 2). Western blot analysis of tumor lysates from *Mdm4*^{Tg1} *p53*$^{+/-}$ indicated that 11 of 13 tumors had high levels of Mdm4 (Fig. 4B). We also selected 13 very well isolated tumors, including seven osteosarcomas, for Southern blot analysis to determine whether these tumors had *p53* loss of heterozygosity (LOH). Strikingly, only 1 of 13 tumors showed *p53* LOH (Fig. 4C) compared with *p53*$^{+/-}$ mice in which approximately half of the tumors showed LOH (30, 34). We also sequenced *p53* from seven tumors, six of which were wild-type for *p53*. One tumor had a Val-to-Ala substitution at p53 amino acid 213, which is not one of the known loss-of-function mutations. These data suggest that overexpression of Mdm4 reduced the selective pressure for inactivating the wild-type *p53* allele in *Mdm4*^{Tg1} *p53*$^{+/-}$ tumors.

Overexpression of Mdm4 dampened p53 response after ionizing radiation

The generation of mice with high levels of Mdm4 offered the opportunity to examine the effect on p53-mediated DNA damage response *in vivo*. To examine the effects of Mdm4 overexpression after DNA damage *in vivo*, we irradiated *Mdm4*^{Tg15} female mice. After ionizing radiation (IR), Mdm4 protein levels decreased in spleens of *Mdm4*^{Tg15} mice, consistent with previous *in vitro* data (refs. 23, 28; Fig. 5A). Additionally, p53 was stabilized to a much lower extent in spleen samples of these transgenic mice than in

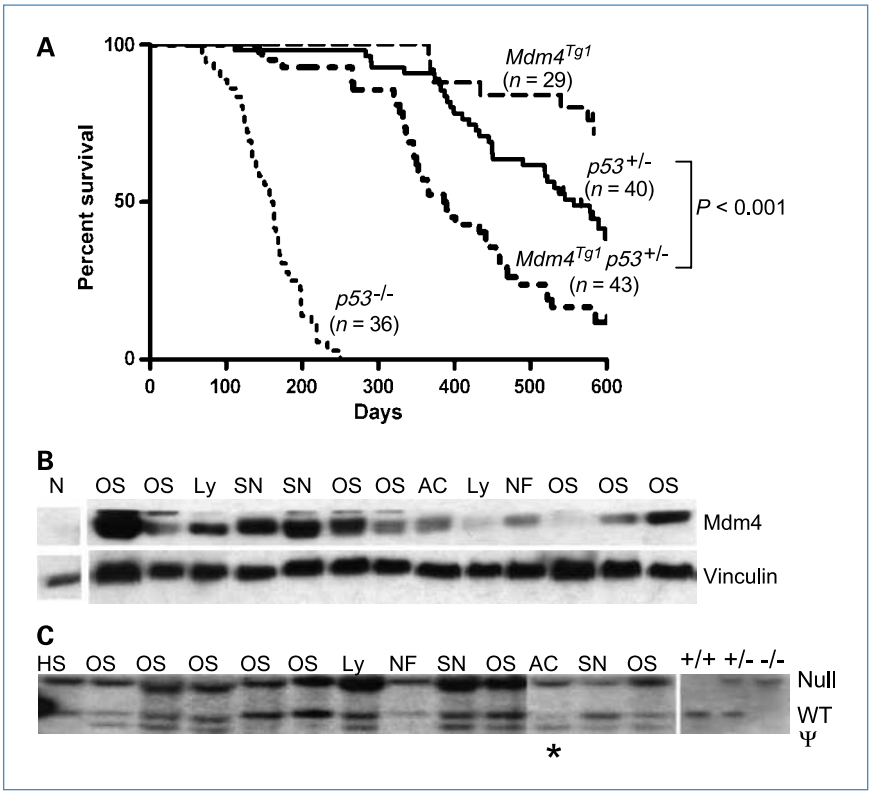

Figure 4. Acceleration of tumorigenesis in *p53*$^{+/-}$ mice by overexpression of Mdm4. A, Kaplan-Meier survival curves for *Mdm4*^{Tg1}, *p53*$^{+/-}$, *Mdm4*^{Tg1} *p53*$^{+/-}$, and *p53*$^{-/-}$ mice. B, Western blots of *Mdm4*^{Tg1}*p53*$^{+/-}$ tumor lysates using Mdm4 and vinculin antibodies. The first lane is a *Mdm4*$^{-/-}$ *p53*$^{-/-}$ MEF control. C, Southern blot analysis of *Mdm4*^{Tg1} *p53*$^{+/-}$ tumor DNA samples. *, sample with LOH. HS, histiocytic sarcoma; OS, osterosarcoma; Ly, lymphoma; NF, neurofibrosarcoma; SN, sarcoma, not otherwise specified; AC, mammary adenocarcinoma; Ψ, p53 pseudogene. *p53* homozygous (+/+), heterozygous (+/–), and null (–/–) tail DNAs were used as controls for the Southern blot.

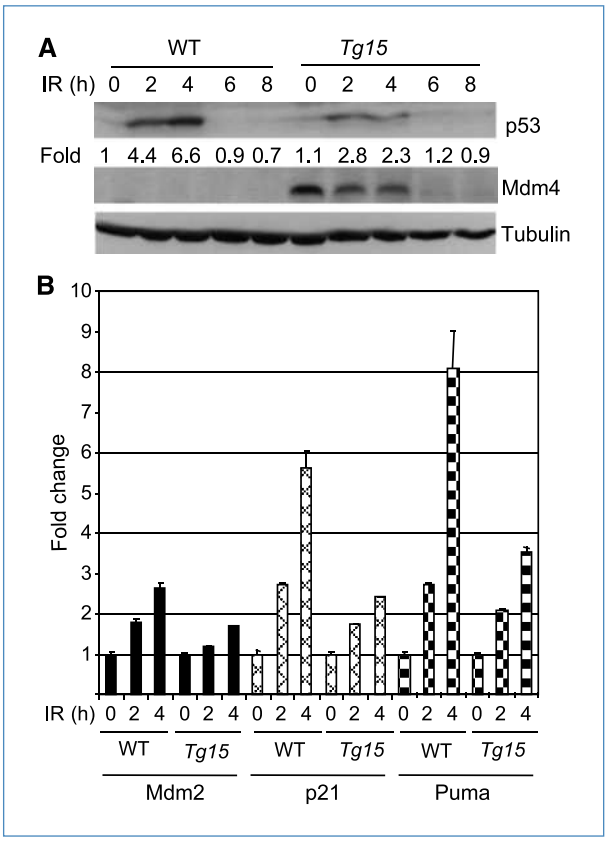

Figure 5. Overexpression of Mdm4 suppresses p53 response after IR. A, a time course of p53 and Mdm4 protein levels in spleens of 6-week-old female mice irradiated with 6 Gy. Tubulin was used as loading control. B, activation of p53 transcriptional targets *Mdm2, p21*, and *Puma* as measured by RT-qPCR in splenocytes from WT and $Mdm4^{Tg15}$ mice after 6 Gy IR. Three mice were examined at each time point. h, hours.

wild-type littermates (Fig. 5A; Supplementary Fig. S2). RT-qPCR experiments for activation of p53 downstream target genes showed that *Mdm2, p21*, and *Puma* mRNA levels (35, 36) were induced to a lesser extent than in wild-type littermates after IR in splenocytes (Fig. 5B). These data suggest that *Mdm4* transgenic mice exhibited a dampened p53 response after IR.

Discussion

We generated transgenic mice with widespread expression of Mdm4. All transgenic lines with Mdm4 overexpression were viable and did not show obvious developmental defects. The two transgenic lines examined, $Mdm4^{Tg1}$ and $Mdm4^{Tg15}$, developed spontaneous tumors (Table 2). This is direct evidence that Mdm4 is a bona fide oncogene that induces tumorigenesis *in vivo*. While $Mdm4^{Tg1}$ tumors had high levels of Mdm4, they also had high levels of Mdm2 and p53. High Mdm2 levels are incompatible with p53 stability as Mdm2 is an E3 ubiquitin ligase for p53. Mdm4 cannot degrade p53 but can block Mdm2 access to p53 as they bind the same domain of p53 with similar affinities (37). Notably, Mdm4 overexpression in normal adult tissues in $Mdm4^{Tg1}$ mice is patchy, but expression of Mdm4 in tumors was high, suggesting that tumors arose from Mdm4-overexpressing cells.

The p53-mediated DNA damage response was also dampened in Mdm4-overexpressing cells. After IR treatment, although Mdm4 levels in $Mdm4^{Tg15}$ mice decreased with time (probably as a cellular response to activate p53), it remained higher than normal at early time points and suppressed transcription of *Mdm2, p21*, and *Puma* in splenocytes. Compared with wild-type mice, mice overexpressing Mdm4 also showed reduced p53 stabilization after IR. These data are consistent with recently published data showing that degradation of Mdm4 after IR is crucial for p53-mediated radiation response (28). High Mdm4 levels may delay the phosphorylation of p53, and therefore hinder p53 stabilization and activation after IR. Because the level of Mdm4 protein affects the radiation response, it may also affect the outcome of radiotherapy in cancer patients.

The median survival of $Mdm4^{Tg1}p53^{+/-}$ mice at 388 days was significantly shorter than that of $p53^{+/-}$ mice at 578 days. $Mdm4^{Tg1}p53^{+/-}$ mice also had significantly decreased lymphoma incidence and increased carcinoma incidence compared with $p53^{+/-}$ mice alone. These data indicate that Mdm4 overexpression not only cooperated with *p53* heterozygosity to accelerate tumorigenesis but also altered tumor spectra. More importantly, Mdm4 overexpression in $p53^{+/-}$ mice allowed retention of wild-type *p53* in most tumors, suggesting that overexpression of Mdm4 reduced the selective pressure to inactivate the wild-type *p53* allele.

Because blocking the inhibitory effects of Mdm4 is a clear therapeutic strategy for cancers with high Mdm4 levels and wild-type *p53* (38), our Mdm4 transgenic mice will be invaluable for testing the efficacy of Mdm4 inhibitors *in vivo*. Because human retinoblastomas express high levels of MDM4 and $Mdm4^{Tg1}$ mice overexpress Mdm4 in the retina, it may be a great model to study the progression of the disease on inactivation of other members of the Rb pathway (18). Also, overexpression of Mdm4 inhibits the therapeutic effect of the Mdm2 inhibitor Nutlin3 (39, 40), indicating that this mouse model can also be useful to examine how overexpression of Mdm4 affects the efficacy of Mdm2 inhibitors.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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