Tumor Susceptibility and Apoptosis Defect in a Mouse Strain Expressing a Human p53 Transgene

Crissy Dudgeon,1 Calvina Kek,2 Oleg N. Demidov,2 Shin-ichi Saito,1 Kenneth Fernandes,3 Alexandra Diot,3 Jean-Christophe Bourdon,3 David P. Lane,1 Ettore Appella,1 Albert J. Fornace, Jr.,1 and Dmitry V. Bulavin2

1Laboratory of Cell Biology, Center for Cancer Research, National Cancer Institute, NIH, Bethesda, Maryland; 2Institute of Molecular and Cell Biology, Singapore; and 3Department of Surgery and Department of Molecular Oncology, Cancer Research UK Cell Transformation Research Group, University of Dundee, Ninewells Hospital, Dundee, Scotland, United Kingdom

Abstract

Activation of apoptosis is believed to be critical for the role of p53 as a tumor suppressor. Here, we report a new mouse strain carrying a human p53 transgene in the mouse p53-null background. Expression of human p53 in these mice was comparable with wild-type murine p53; however, transactivation, induction of apoptosis, and G1-S checkpoint, but not transrepression or regulation of a centrosomal checkpoint, were deregulated. Although multiple functions of p53 were abrogated, mice carrying the human p53 transgene did not show early onset of tumors as typically seen for p53-null mice. In contrast, human p53 in the p53-null background did not prevent accelerated tumor development after genotoxic or oncogenic stress. Such behavior of human p53 expressed at physiologic levels in transgenic cells could be explained by unexpectedly high binding with Mdm2. By using Nutlin-3a, an inhibitor of the interaction between Mdm2 and p53, we were able to partially reconstitute p53 transactivation and apoptosis in transgenic cells. Our findings indicate that the interaction between p53 and Mdm2 controls p53 transactivation, G1 arrest (12), maintenance of genomic integrity, and tumorigenesis. (Cancer Res 2006; 66(6): 2928-36)

Introduction

Although the p53 gene is inactivated through mutation in approximately half of all human tumors (1), p53 function is believed to be disrupted in most tumors, including those with wild-type (WT) p53 (2). It is generally accepted that the function of p53 as a tumor suppressor relies on its ability to activate apoptosis (3, 4) through the ability of p53 to activate transcription of proapoptotic genes (5, 6). Increased expression of proapoptotic proteins, such as Bax, Noxa, Puma, PERP, and p53Aip1, results in cytochrome c release and activation of multiple caspases, ultimately inducing apoptosis. The dependence of p53 on its transactivation potential in apoptosis activation was confirmed using mouse cells in which the endogenous p53 gene had been replaced with a p53 (L25Q and W26S) mutant (7). This mutant lacks the ability to activate various p53 target genes, except for the Bax gene. Accumulation of p53 in the cytosol and its translocation to the mitochondria may also contribute to the activation of apoptosis (8, 9). In this case, the p53 protein can directly permeabilize the outer mitochondrial membrane by forming complexes with Bcl-XL and Bcl-2 proteins or function analogously to the BH3-only subset of proapoptotic Bcl-2 proteins to activate Bax and trigger apoptosis. Interestingly, p53 binding to Bcl-XL is regulated via its DNA-binding domain, and tumor-derived transactivation-deficient mutants of p53 lose the ability to interact with Bcl-XL and promote apoptosis. Thus, the DNA-binding domain of human p53 is critical for activation of apoptosis via direct activation of transcription of proapoptotic genes and its transcription-independent functions.

In agreement with a critical role of p53 in regulation of tumorigenesis, p53-null mice develop spontaneous tumors from age 6 to 10 months (10, 11). Although cells established from p53-null mice have a variety of abnormalities, including defects in apoptosis activation, G1 arrest (12), maintenance of genomic integrity, and centrosome duplication (13), the cause of spontaneous tumorigenesis in p53-null mice remains poorly understood. In the present work, we generated a transgenic mouse strain carrying a human p53 transgene that was back-crossed into the mouse p53-null background. This mouse model (hereafter SWAP for human p53 swapped for mouse p53) allowed us to determine whether human p53 could functionally replace mouse WT p53 to prevent spontaneous and induced tumor formation as well as to generate a tool for subsequent analysis of human p53 functions under various physiologic conditions. Surprisingly, we found that human p53 that expressed at physiologic levels is transcriptionally deficient in mouse cells and as a result is unable to activate ionizing radiation (IR)–induced apoptosis and the G1-S checkpoint, although p53 exhibited apparently normal accumulation and post-translational modifications after DNA damage. Although activation of apoptosis and the G1-S checkpoint were deregulated, mice carrying the human p53 transgene did not show accelerated spontaneous tumorigenesis as typically seen and as reported previously for p53-null mice. In contrast, human p53 failed to prevent accelerated tumor formation in mice treated with IR or crossed with Eμ-myeloma transgenic mice. Such behavior of human p53 expressed at physiologic levels in mouse cells could be explained by unexpectedly high binding with Mdm2. The addition of Nutlin-3a, an inhibitor of Mdm2-p53 binding, could partially reconstitute p53 transactivation and apoptosis in SWAP cells. Our findings indicate that the role of p53 in spontaneous tumorigenesis does not fully rely on its functions as a regulator of apoptosis as well as its ability to transcriptionally activate typical p53-regulated genes, and our mouse model provides a new in vivo tool to further dissect this critical function of human p53.
Materials and Methods

Generation of SWAP mice. All animal protocols used in this study were approved by the National Cancer Institute Animal Safety and Use Committee. Human genomic p53 subclones were kindly provided by Dr. P. Chumakov (Institute of Molecular Biology, Moscow, Russia). To generate a multicopy transgene, we used a genomic fragment containing ~20 kb of human genomic DNA beginning at the transcription start site and ending at the 881G site in the genomic sequence outside the p53 coding sequence. Transgenic mice from two separate founders were back-crossed into a mixed p53-null background. The presence of a human p53 transgene was confirmed in a PCR (primers available upon request).

Sequencing of human p53 in SWAP mice. Two 9-week-old WT mice were sacrificed using CO2. Their kidney and thymus were removed, placed in Trizol (Invitrogen, Carlsbad, CA), and homogenized using a Turrax T25 Basic Disperser (IKA Labortechnik, Staufen, Germany) at 24,000 rpm. RNA was extracted according to the manufacturer’s instructions. Kidney and thymus cDNA was prepared using a reverse transcription-PCR (RT-PCR) kit (Invitrogen) with random hexamers. DNA corresponding to the p53 sequence was amplified with specific primers (sequences available upon request) and sequenced.

Quantitative p53 expression analysis. Nine-week-old WT and SWAP mice were sacrificed by CO2 asphyxiation. Tissues were removed, placed in Trizol (Invitrogen), and homogenized using a turrax T25 Basic Disperser (IKA Labortechnik, Staufen, Germany) at 24,000 rpm. RNA was extracted using the manufacturer’s instructions. Kidney and thymus cDNA was prepared using a reverse transcription-PCR (RT-PCR) kit (Invitrogen) with random hexamers. DNA corresponding to the p53 sequence was amplified with specific primers (sequences available upon request) and sequenced.

Luciferase reporter assay. Early-passage WT, p53-null, and SWAP MEFs were transfected with p53-luciferase reporter (Stratagene, Cedar Creek, TX) and treated with 10 μM Nutlin-3a overnight. Luciferase was quantified using the dual-luciferase kit (Promega, Madison, WI).

Results

To generate a human p53 transgene, an ~20-kb fragment corresponding to human genomic p53 sequence from exon 1 to the polyadenylation signal was used to generate a multiple copy human p53 transgene. This sequence lacks the promoter upstream of exon 1 but contains the second promoter within intron 1. The promoter inside intron 1 is much stronger than the upstream promoter and does not have any negative regulating elements, which are located in the upstream promoter (17). Two independent mouse founders were established. Southern blot analysis confirmed the copy number for the p53 transgene to be 20 to 30 copies in each founder (data not shown). The transgenic human p53 mice were then back-crossed into a mouse C57BL/J, 129SvJ mixed p53-null background. All genotypes were confirmed by PCR using specific primers, and the WT sequence of the human p53 transgene was confirmed by sequencing cDNA purified after reverse transcription of RNA obtained from tissues of SWAP mice (data not shown). Furthermore, we confirmed that SWAP p53 has a WT conformation as it could be immunoprecipitated with the WT (Ab1620) but not the mutant conformational antibody (Ab240; data not shown). For this transgene, we used an Arg22 polymorphic form of human WT p53. This polymorphic form has been shown to induce apoptosis to a
greater extent than the Pro$^{72}$ form (18). Therefore, because it is a stronger inducer of apoptosis, we expected to see apoptosis at normal or above-normal levels.

We determined whether the p53 protein levels in transgenic mice were similar to those of WT mouse p53. WT mouse p53 protein and human p53 protein from transgenic mice were blotted from different tissues using an antibody that detects both mouse and human p53, Pab421 (Fig. 1A). The expression of human p53 in SWAP mice was similar to the expression of endogenous mouse p53 among different tissues; however, several other bands for human p53 were detected on darker exposure. The latter are consistent with the fact that multiple splice forms exist for human p53 (18). As there are at least nine putative p53 isoforms known to date, we tested organs from SWAP mice for the presence of isoforms using a real-time PCR procedure (19). We found that with exception for p53γ, Δ40p53γ, and Δ133p53γ all other six remaining isoforms were present in SWAP mice (data not shown). This pattern of expression was observed in both founders, thus allowing us to assume that both transgenic lines would be identical in p53 response.

Transgenic SWAP mice were fertile and developed normally. A survival curve was plotted to determine the median life span (MLS). As shown in Fig. 1B, SWAP mice lived substantially longer than p53-null mice, and had a MLS of 9.5 months ($n = 17$) compared with 5 months for p53-null mice ($n = 21$). A similar MLS of 9.5 months has been reported for another mouse strain, in which a murine p53 transgene was generated and then back-crossed into the p53-null background (20). We observed tumor formation in some transgenic p53 mice: Five thymic lymphomas and 3 lymphomas of different locations were observed in a group of 17 mice. The remaining mice died with no visible tumors, and a full necropsy on multiple mice did not reveal a cause of death.

To investigate whether tumors had arisen as a result of loss of p53 expression, we analyzed human p53 levels in thymic lymphomas. Loss or down-regulation of human p53 expression was confirmed in all four tested thymomas (Fig. 1C), arguing that human p53 behaved as an endogenous replica of mouse p53 in preventing spontaneous tumor formation and that spontaneous tumors developed only after the loss or down-regulation of p53 expression.

Given that one of the important functions of p53 as a tumor suppressor is its ability to activate apoptosis (3, 4), we analyzed the apoptotic response to IR in SWAP mice. WT and SWAP mice were irradiated with either 3 or 4 Gy, and 4 hours later, the thymus and spleen were removed and p53 levels were analyzed. As shown in Fig. 2A, p53 levels increased dramatically in response to IR in both WT and SWAP splenocytes and thymocytes. These data argue that the mechanisms that regulate p53 stabilization after IR are fully functional in both cases. Next, mice were subjected to 4 Gy IR, and both thymocytes and splenocytes were harvested and stained for apoptosis with Annexin V (Fig. 2B). As anticipated, IR induced significant apoptosis in WT mice (58% versus 11% for thymocytes and 32% versus 13% for splenocytes) 4 hours after IR. In contrast, only slight, if any, apoptosis induction was observed in p53-null mice (14% versus 6% for thymocytes and 19% versus 15% for splenocytes). Intriguingly, SWAP thymocytes and splenocytes showed no appreciable activation of apoptosis after IR (8% versus 6% for thymocytes and 15% versus 12% for splenocytes). A similar lack of apoptosis activation was observed in SWAP thymocytes and splenocytes 8 and 12 hours postradiation (data not shown). To further confirm that the defect in apoptotic response was not tissue specific, SWAP females were crossed with p53$^{+/+}$ males. At day 11.5 of pregnancy, these mice were irradiated with 4 Gy IR, and 4 hours later, embryos were extracted, genotyped, and stained using the BrdU/TdT-FragEL kit to detect 3'-OH ends of DNA fragments generated in response to apoptotic signals (Fig. 2C). Similar to other tissues, we did not observe substantial activation of apoptosis in the embryonic tissues of SWAP mice. These data argue that the transgenic human p53 lacks the ability to activate IR-induced apoptosis in vivo in mice.

As p53-dependent apoptosis is required to prevent IR-induced tumorigenesis (21), we next tested whether IR (4 Gy) can expedite tumor formation in SWAP mice. In this experiment, an estimated MLS for irradiated 8- to 12-week-old SWAP mice was determined to be 5.5 months ($n = 21$) compared with the >1 year MLS for irradiated WT mice ($n = 15$; Fig. 3A). The survival of irradiated SWAP mice was similar to that reported previously for irradiated p53-null mice (~5 months; ref. 22). We further analyzed the levels of human p53 in three thymic lymphoma samples and found that it remained at levels comparable with the normal nonirradiated thymus (Fig. 3B). These data suggest that the p53 function as an activator of apoptosis is essential in the prevention of tumor formation after IR.
To determine whether transgenic p53 can prevent tumor formation in the presence of activated oncogenes, we crossed both SWAP and p53-null mice with Eμ-myc transgenic mice. As reported previously, tumor formation in Eμ-myc transgenic mice is dependent on p53 function as an inducer of apoptosis (23). The resulting progeny had a genotype of Eμ-myc SWAP p53+/- and Eμ-myc p53+/-, and we anticipated rapid tumor formation in p53+/- mice as a result of the loss of a second copy of the p53 gene [loss of heterozygosity (LOH)]. We found that survival of Eμ-myc SWAP+/- (n = 16) and Eμ-myc p53+/- (n = 11) was similar, with a MLS of 47 and 51 days, respectively (Fig. 3C). LOH of mouse p53 in tumor samples was confirmed by PCR (data not shown). Analysis of human p53 protein from Eμ-myc SWAP lymphomas showed the loss of expression in one of the samples (Fig. 3D). However, most tumors displayed the same or slightly higher levels of human p53. These data suggest that human SWAP cannot prevent tumor formation in the presence of activated Myc, emphasizing the critical role of p53 in the activation of apoptosis to prevent Myc-induced tumorigenesis.

Because human p53 is deficient in apoptosis activation in our mouse model (Fig. 2), we determined whether upstream kinases that activate mouse p53 during genotoxic stress were able to

Figure 2. Transgenic human p53 is defective in the activation of apoptosis in vivo after γ-irradiation. A, human p53 expression in γ-irradiated WT and SWAP mice was analyzed 4 hours postirradiation in both thymus and spleen. Control samples were from nonirradiated mice. The level of actin was used as a loading control. B, in vivo apoptosis analysis of thymocytes and splenocytes was carried out 4 hours after irradiation with 4 Gy for WT (top), p53-null (middle), and SWAP (bottom) mice using Annexin V staining (see Materials and Methods). C, in vivo apoptosis analysis of irradiated p53+/- and SWAP embryos (limb bud shown) was done using a BrdUTP-TdT FragEL kit.

Figure 3. Transgenic human p53 does not prevent tumor formation in IR-irradiated mice and in the presence of the Myc oncogene. A, survival curve of SWAP and WT mice irradiated with 4 Gy IR was analyzed and MLS was estimated as 5.5 months for SWAP and >1 year for WT mice. B, human p53 protein expression in thymic lymphomas obtained from SWAP mice was analyzed as described in Materials and Methods. N, normal thymic tissue; T, individual mouse thymic lymphomas. The level of actin was used as a loading control. C, in vivo apoptosis analysis of irradiated p53+/- and SWAP embryos (limb bud shown) was done using a BrdUTP-TdT FragEL kit.
phosphorylate human p53 in MEFs. As shown in Fig. 4A, phosphorylation on Ser15, Ser33, Ser46, and Ser392 was rapidly induced after IR or UV irradiation in SWAP MEFs. Hence, murine kinases responsible for phosphorylation (and presumably activation) of mouse p53 could target human p53. As the upstream events in activation of human p53 seemed to be functional, we analyzed checkpoint activation. Analysis of IR-induced G1-S checkpoint by counting the number of cells labeled with BrdUrd revealed that human p53 was not capable of restoring this arrest in SWAP MEFs (Fig. 4B).

One critical function of p53 is to maintain genomic stability, which includes a tight control of centrosome number. We challenged SWAP+/−, p53-null, and SWAP MEFs with 1 mmol/L hydroxyurea and counted the number of centrosomes. As expected, the lack of p53 in MEFs caused amplification of centrosomes in the presence of hydroxyurea (Fig. 4C). Interestingly, human p53 in a mouse background was able to maintain the normal number of centrosomes in the presence of hydroxyurea, similar to SWAP+/−, but significantly different from p53-null MEFs (Fig. 4C). To address if the hydroxyurea was initiating a G1-S-phase arrest in treated cells, flow cytometry was used on fixed cells stained with propidium iodine (Fig. 4D). In untreated SWAP+/− cells, 58% were in G0-G1 phase and 19% in S phase. As expected after hydroxyurea treatment, 92% of cells were in G0-G1 phase and 4% in S phase. p53-null cells did not initiate an arrest, having 29% of cells before and 37% of cells after hydroxyurea treatment in G0-G1 phase and 32% and 28% before and after hydroxyurea addition in S phase. SWAP cells initiated only partial arrest, with 38% before hydroxyurea treatment and 47% after hydroxyurea treatment in G0-G1 phase and 49% and 20% before and after hydroxyurea treatment in S phase.

To test human p53 transcriptional activation in SWAP mice, quantitative mRNA analysis was done using a dot-blot procedure or real-time PCR. We determined the levels of p53-regulated genes involved in cell cycle control [Gadd45a, XPC, and Cdkn1a (p21/Waf1)] and apoptosis (Bax and Noxa). Figure 5A shows that whereas statistically significant induction of Gadd45a, XPC, and Cdkn1a mRNA was observed in the thymus of WT mice, little induction was detected in SWAP or p53-null mice. Similarly, activation of proapoptotic genes in cultured thymocytes was completely abrogated in SWAP cells after IR compared with substantial induction in WT cells (Fig. 5B). These results argue that lack of apoptotic and G1-S checkpoint activation in SWAP cells after IR is most likely a consequence of a defect in p53 transactivation of downstream genes.

Contrary to the lack of transcriptional activation, down-regulation of a set of genes, Cdc2, CyclinB1, and Cdc25C, which are regulated in a p53-dependent manner after DNA damage (24), was observed in SWAP splenocytes (Fig. 5C) challenged with IR. Thus, human p53 in a mouse background retains several, but not all, features of mouse WT-p53, such as regulation of centrosome number in the presence of hydroxyurea as well as transrepression activity.
As our SWAP mice seemed to be deficient in the expression of p53 isoforms, it was our expectation that this could be the reason for the inability to transactivate p53-responsive genes. We generated all three p53 isoforms in a pCAG vector and transfected them into MEFs. Although the level of expression of different isoforms was comparable with WT p53 when transfected into p53-null MEFs (Fig. 6A), neither a single p53 isoform nor all three together were able to reconstitute p53-dependent transactivation in SWAP MEFs to the levels observed for WT MEFs (Fig. 6A, right, lane 1). Thus, the lack of p53 isoforms in SWAP mice has no effect on the inability of p53 to transactivate p53-dependent genes.

To understand why human p53 is rendered inactive in SWAP cells, we challenged SWAP MEFs with different types of stress. Only early-passage (passages 2-4) MEFs were used for this study, as it seemed that the level of human p53 in SWAP MEFs declines dramatically after several passages (data not shown). WT, p53-null, and SWAP MEFs were transfected with a reporter plasmid carrying a p53-responsive element that drives luciferase expression (Fig. 6A, right panel). The following day, cells were treated with different agents and analyzed for luciferase activity 24 hours later. Whereas treatment with IR, UVC, doxorubicin, and N-(phosphonacetyl)-L-aspartic acid, serum deprivation, and transfection with p19Arf and oncogenic Ras induced p53 activation in WT MEFs, it had no apparent effect on p53-dependent transcriptional activation in either p53-null or SWAP MEFs (data not shown). The only exception was the treatment of cells with an inhibitor of p53-Mdm2 interaction, Nutlin-3a (25). Inclusion of Nutlin-3a increased p53-dependent transactivation 3.5-fold in WT MEFs and 2.5-fold in SWAP MEFs (Fig. 6B). Further analysis of p53-Mdm2 binding revealed that precipitates with anti-Mdm2 antibody contained substantially more p53 in nonstressed and IR-treated SWAP MEFs compared with WT MEFs (1.6-fold increase in nonstressed SWAP cells and 2.7-fold increase in IR-treated SWAP cells; Fig. 6C). To ask whether this reactivation of p53 in the presence of Nutlin-3a is sufficient to execute p53-dependent effects, we treated thymocyte cultures obtained from WT, SWAP, and p53-null mice with Nutlin-3a or Nutlin-3b (the chimeric inactive form of Nutlin-3a; ref. 25) in the presence of IR (4 Gy). As shown in Fig. 6D (left), treatment of SWAP thymocytes with Nutlin-3a induced p53-dependent apoptosis, which was statistically significant from p53-null cultures starting at 8 hours after IR (P = 0.039, unpaired t test) and was half as much as in WT cells after 16 hours (P = 0.027, unpaired t test). On the other hand, inclusion of Nutlin-3b had no apparent effect on apoptosis activation in both p53-null and SWAP thymocytes treated with IR (Fig. 6D, right). Overall, these data support the notion that a defect in p53 signaling in SWAP mice is due to a stronger binding with Mdm2.

Discussion

The p53 tumor suppressor is one of the key regulators of tumorigenesis and multiple mouse models have been generated to investigate its functions in vivo. The first line of in vivo evidence highlighting the role of p53 as a tumor suppressor came from the characterization of mice where the endogenous copy of mouse p53 had been disrupted by homologous recombination (10, 11). The conclusion from these studies was that p53-null mice develop spontaneous tumors, usually lymphomas and soft tissue sarcomas,
and die within 6 to 10 months. Since then, multiple attempts have been made to clarify the role of p53 in the regulation of spontaneous and induced tumorigenesis. Both in vitro and in vivo evidence has suggested the contribution of the proapoptotic functions of p53 in the inhibition of in vivo tumor formation. However, direct evidence showing that the function of p53 as an inducer of apoptosis is absolutely critical for protection from spontaneous tumorigenesis has not been provided.

As it is critical to understand the role of human p53 in tumorigenesis in an in vivo context, there is a need to generate mouse strains with humanized p53. In previous experiments, homologous recombination was used to introduce the DNA-binding region of human p53, creating a chimera that retained the mouse regulatory regions at the NH2 and COOH termini (26). In that case, human p53 seemed to substitute perfectly for the mouse gene, induced downstream target genes, suppressed tumor formation, and even exhibited the same mutation spectrum for inactivation that occurs in humans (27–29). In our model, using human p53 transgenic mice, we find a complete lack of p53-dependent transcriptional activation and activation of apoptosis after DNA damage in multiple tissues. Although we used an Arg72 polymorphic form of human p53, which has higher proapoptotic activity than another WT homologue (Pro72 polymorphic form; ref. 18), a defect in apoptosis activation was observed, similar to the defect seen in p53-null mice. Despite deregulated apoptosis, SWAP mice lived longer than p53-null mice and developed spontaneous tumors only as a result of the loss of human p53 expression (Fig. 1). Thus, there is a fundamental difference between introducing a p53 transgene in a BAC (this work) and replacing only a part of the p53 gene by homologous recombination. One substantial difference in these two sets of experiments is that in mice the NH2 and COOH termini of human p53 may not interact with regulatory factors in an appropriate fashion. Indeed, we find the interaction of human p53 with mouse Mdm2 in our transgenic mice increased and disruption of this interaction with the chemical compound Nutlin-3a (25) increases the ability of transgenic p53 to activate target genes and induce apoptosis. Importantly, although inclusion of Nutlin-3a did increase p53-dependent transactivation and apoptosis in SWAP cells, it did not restore its levels to the ones observed for WT cells (Fig. 6B and D).

Figure 6. Increased Mdm2 binding in SWAP cells, not the absence of p53 isoform expression, causes lack of p53 transactivation. A, p53-null MEFs were transfected with either WT p53 or different p53 isoforms and the level of expression was analyzed using Ab-7 antibody (left panel). SWAP MEFs were cotransfected with a reporter plasmid carrying a p53-responsive element and either pCAG (lane 1 for WT and lane 2 for SWAP MEFs), WT p53 (lane 3), or different p53 isoforms (lanes 4–7). The luciferase activity was analyzed 24 hours later (right panel). B, WT, p53-null, and SWAP MEFs were transfected with p53-luciferase reporter and treated with Nutlin-3a (10 μmol/L) overnight. Luciferase activity was normalized to the values determined for nontreated WT-MEFs. Total p53 level was analyzed using Ab-7 antibody. C, WT and SWAP MEFs were treated with 4 Gy irradiation and treated with 20 μmol/L ALLN 4 hours before harvest. Protein extracts were immunoprecipitated with an anti-Mdm2 (SMP-14) antibody. The levels of p53 and Mdm2 were analyzed using specific antibody. D, cultured thymocytes obtained from WT, p53-null, and SWAP mice were pretreated with 12 μmol/L Nutlin-3a (left) or 12 μmol/L Nutlin-3b (right) for 4 hours and then treated with IR for the designated times. Cells were collected at different time points and analyzed for apoptosis using an Annexin V staining protocol. Asterisks, time points of significant value.

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Mdm2-dependent degradation of p53, as the levels of p53 in the interaction of Mdm2 and p53 is expected to be somewhat complex formation between both proteins. Importantly, although is expected that mutation of this site to alanine could facilitate the regulation of the p53 interaction with Mdm2 (31–33); thus, it our data, Chk2-deficient mice show complete lack of activation of rate of spontaneous tumorigenesis.

Phosphorylation of mouse Ser23 (human Ser20) on p53 has been substituted to alanine, the p53 S23A knock-in mice (30). The reason for this apparently reduced efficiency of Nutlin-3a rather inhibit its transcriptional activity (35). In our mouse model, although human p53 did not have any mutations and retained structurally intact WT conformation, the interaction with mouse Mdm2 also was elevated and p53 was transcriptionally inactive. As a result of these molecular changes, both the mouse S23A knock-in mice and our strain showed reduced activation of apoptosis in the thymus and embryonic tissues. However, this was not sufficient to induce spontaneous thymic lymphomas, suggesting that the function of p53 as an activator of apoptosis is not critical for an early outbreak of cancer.

Recent data using Puma and Chk2 knockout mice further support the fact that neither activation of apoptosis nor p53 function as a transcriptional activator are critical for spontaneous thymic lymphoma development (15, 36). Puma has been suggested as one of the key downstream regulators of p53-dependent apoptosis in both mouse and human cells (36–38). Inactivation of Puma in mice by homologous recombination resulted in a significant attenuation of radiation-induced apoptosis in different cell types. However, Puma−/− mice did not show an accelerated rate of spontaneous tumorigenesis in vivo (36). Consistent with our data, Chk2-deficient mice show complete lack of activation of p53-responsive genes after IR, yet mice are rendered resistant to spontaneous tumors (15). Hence, other functions of p53, such as a direct protein-protein interaction or transcriptional repression, regulate the ability of p53 to suppress early onset of certain types of cancer (for review, see ref. 12).

The potential role of p53 in controlling genomic stability could be especially important in regulating spontaneous tumorigenesis in the absence of p53. Recent data by Liu et al. suggested that the ability of p53 to retain chromosomal stability, but not apoptosis activation, could be critical for suppression of early-onset tumorigenesis (39). In light of our data, it seems reasonable to speculate that the mechanism(s) regulating the hydroxyurea-like induced centrosome amplification checkpoint, which ultimately may contribute to regulation of chromosomal stability, may be essential in controlling spontaneous tumorigenesis. It is uncertain which function of p53 is critical for the regulation of centrosomal checkpoint and suppression of centrosome amplification under conditions when DNA synthesis is inhibited. Some data point toward the ability of WT human p53, but not the DNA-binding domain mutants, to interact with centrosomal proteins. In agreement with this notion and using microcapillary high-performance liquid chromatography tandem mass spectrometry analysis, we found several centrosomal proteins bound to both mouse (from WT mice) and human (from SWAP mice) p53: nucleophosmin, α-tubulin, vimentin, pericentrin 2 (kendrin), and ODF2 (data not shown). Thus, it is possible that p53 could be a part of a centrosomal protein complex, whose assembly is essential in the regulation of a ‘centrosomal’ checkpoint. In turn, proper regulation of a centrosomal checkpoint could be critical to maintain chromosomal stability and thus suppress spontaneous tumorigenesis.

In summary, our transgenic mice and cells established from them allowed us to distinguish the role of p53 in apoptosis and checkpoint controls versus its role in spontaneous tumorigenesis, providing further insight into the multiple functions of p53.

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Grant support: Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer Research, and Agency for Science, Technology and Research (Singapore; C. Keck, O.N. Demidov, D.P. Lane, and D.V. Bulavin). Nutlin-3a and Mdm2 also was elevated and p53 was transcriptionally inactive. As a result of these molecular changes, both the mouse S23A knock-in mice and our strain showed reduced activation of apoptosis in the thymus and embryonic tissues. However, this was not sufficient to induce spontaneous thymic lymphomas, suggesting that the function of p53 as an activator of apoptosis is not critical for an early outbreak of cancer.

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The reason for this apparently reduced efficiency of Nutlin-3a in vivo is uncertain; it could be a result of an existence of additional molecules that can maintain Mdm2 in a complex with p53 even when Nutlin-3a is present. This possibility is currently under detailed investigation.

Our mouse data agree with another mouse strain in which Ser23

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