Cancer Research

Mouse Models for the p53 R72P Polymorphism Mimic Human Phenotypes

Feng Zhu[1], Martijn E.T. Dollé[3], Thomas R. Berton[1], Raoul V. Kuiper[3,5], Carrie Capps[1,4], Alexsandra Espejo[1], Mark J. McArthur[2,4], Mark T. Bedford[1,4], Harry van Steeg[3], Annemieke de Vries[3], and David G. Johnson[1,4]

Abstract

The *p53* tumor suppressor gene contains a common single nucleotide polymorphism (SNP) that results in either an arginine or proline at position 72 of the p53 protein. This polymorphism affects the apoptotic activity of p53 but the mechanistic basis and physiologic relevance of this phenotypic difference remain unclear. Here, we describe the development of mouse models for the p53 R72P SNP using two different approaches. In both sets of models, the human or humanized p53 proteins are functional as evidenced by the transcriptional induction of p53 target genes in response to DNA damage and the suppression of early lymphomagenesis. Consistent with *in vitro* studies, mice expressing the 72R variant protein (p53R) have a greater apoptotic response to several stimuli compared with mice expressing the p53P variant. Molecular studies suggest that both transcriptional and nontranscriptional mechanisms may contribute to the differential abilities of the p53 variants to induce apoptosis. Despite a difference in the acute response to UV radiation, no difference in the tumorigenic response to chronic UV exposure was observed between the polymorphic mouse models. These findings suggest that under at least some conditions, the modulation of apoptosis by the R72P polymorphism does not affect the process of carcinogenesis. *Cancer Res; 70(14); 5851–9. ©2010 AACR.*

Introduction

Mouse models have been instrumental in contributing to our understanding of how rare, inherited mutations can cause some human diseases. However, the development of many diseases, including cancer, often involves the interaction of relatively common polymorphisms combined with specific environmental insults. At present, it is unclear whether common human genetic variations can be modeled phenotypically in the mouse.

The *p53* tumor suppressor gene contains a nonsynonymous single nucleotide polymorphism (SNP) that results in either an arginine (R) or proline (P) at position 72 of the p53 protein (Supplementary Fig. S1). The allelic distribution of this common SNP varies in different ethnic backgrounds and geographic locations. For example, the P-encoding allele is more prevalent in African populations, whereas the R-encoding allele is more common in Caucasians (1). A large number of epidemiologic studies have associated this SNP with risk for developing various cancers but different genotypes are associated with predispositions to different cancers, and in some cases, there is conflicting data (2).

Consistent with the epidemiologic data, laboratory evidence suggests that the p53 polymorphic variants have different biochemical and biological properties. The R72P polymorphism results in a nonconservative amino acid change that alters the structure of p53, as evidenced by a difference in protein migration on SDS-PAGE. No differences in protein induction, stability, or DNA binding properties have been noted between the two p53 variants. Nonetheless, the variant encoding R at codon 72 (p53R) is a more potent inducer of apoptosis and inhibitor of oncogenic transformation *in vitro* (3). Despite the general agreement that p53R promotes apoptosis more efficiently than p53P, the molecular mechanism underlying this functional difference is controversial. Some studies suggest the two variants differ in their abilities to transcriptionally activate certain target genes (3–5). Other studies suggest that the difference in apoptotic potential between the two variants

Authors' Affiliations: [1]The University of Texas M.D. Anderson Cancer Center, Department of Carcinogenesis, Science Park-Research Division, Smithville, Texas; [2]The University of Texas M.D. Anderson Cancer Center, Department of Veterinary Sciences, Bastrop, Texas; [3]Laboratory for Health Protection Research (pb 12), National Institute of Public Health & Environment, Bilthoven, the Netherlands; [4]The University of Texas Graduate School of Biomedical Sciences at Houston, Houston, Texas; and [5]Dutch Molecular Pathology Center, Department of Pathobiology, Faculty of Veterinary Medicine, Utrecht University, Utrecht, the Netherlands

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

Corresponding Authors: David G. Johnson, The University of Texas M.D. Anderson Cancer Center, Department of Carcinogenesis, Science Park-Research Division, P.O. Box 389, Smithville, TX 78957. Phone: 512-237-9511; Fax: 512-237-6533; E-mail: djohnson@mdanderson.org and Annemieke de Vries, Laboratory for Health Protection Research (pb 12), National Institute of Public Health & Environment, A. van Leeuwenhoeklaan 9, 3721 MA Bilthoven, the Netherlands. E-mail: Annemieke.de.Vries@rivm.nl.

doi: 10.1158/0008-5472.CAN-09-4646

is independent of transcriptional regulation and instead involves a difference in mitochondrial localization (6, 7). It should be noted that these previous studies were all performed under *in vitro* conditions involving p53 overexpression, temperature-sensitive mutants, and/or genetically unmatched cell lines. This has led to uncertainty regarding the biological relevance of the R72P polymorphism, and thus, there is a need to develop models in which this SNP can be studied under more defined and physiologic conditions.

Here, we describe the development of mouse models for the p53 R72P polymorphism using two different approaches. We find that the human p53 variants or chimeric p53 proteins containing human exon 4 sequences are functional in mice and are responsive to various forms of DNA damage. Moreover, we find that the p53 variants display differences in their abilities to induce apoptosis, which may involve both transcriptional and nontranscriptional mechanisms. These findings serve as proof of principle that common human genetic variations can be modeled in the mouse, allowing detailed functional analysis of effects of the variations on molecular and cellular processes *in vivo*.

Materials and Methods

Generation of human *p53* bacterial artificial chromosome transgenic mouse lines

The human bacterial artificial chromosome (BAC) clone 66H22 was obtained from Research Genetics and contains the entire *p53* gene locus encoding arginine at codon 72, including 7 Kb of upstream sequence (Supplementary Fig. S2). To introduce the polymorphic variant into the BAC clone, a technique for generating point mutations in BACs by homologous recombination in bacteria was used (8). BAC DNA, containing the original sequences encoding p53R or the modification encoding p53P, was purified, and the entire 98-Kb insert was isolated following digestion with *Not*1. Transgenic founders were generated in an FVB strain background by pronuclear injection, and Southern blot analysis using a human-specific probe identified founders. Low copy number founders were used to establish lines, and offsprings were further analyzed by a quantitative real-time PCR assay to identify transgenic lines with a single copy of the human BAC transgene. One line for each p53 variant (p53R #4 and p53P #7) was chosen for further analysis.

Generation of human *p53* exon 4 knock-in mouse lines

Two targeting constructs were made to humanize exon 4 exclusively and leave the original surrounding intronic sequences intact (Supplementary Fig. S3). These constructs were based on existing vectors containing the mouse *p53* gene with a Li-Fraumeni mutation in exon 8 (R270H; refs. 9, 10). Site-directed mutagenesis was used to generate the polymorphic variant in human exon 4. The genetic distance between exon 4 and exon 8 was sufficient to allow targeting of exon 4 without the inclusion of the mutant exon 8. Embryonic stem (ES) cell clones containing correctly targeted human exon 4 sequences were identified by Southern blot analysis, and the presence of the polymorphism was verified by PCR and digestion with *Btg1* (Supplementary Fig. S4). Blastocyst injections were performed with ES cell clones containing one or the other human exon 4 variant and lacking the R270H mutation. Deletion of the transcriptional stop cassette containing the pGK-puromycin selectable marker was accomplished by crossing knock-in mice to CAG-Cre deleter mice. For UV studies, knock-in mice encoding either p53R or p53P were backcrossed to the SKH hairless strain for four generations to facilitate chronic UVB exposure.

UV and IR treatments

An irradiation chamber containing 12 Westinghouse FS20 sunlamps and a rotating platform was used as the UVB source to irradiate BAC transgenic mice (11). Similarly, knock-in mice were UVB irradiated in a chamber containing Phillips TL12 lamps (12). Westinghouse FS20 sunlamps that generate primarily UVB were also used for treatment of primary mouse adult fibroblasts (MAF). An RS 2000 X-ray Irradiator (Rad. Source) was used for IR treatment of mice and primary cells. All mouse treatments have been approved by The University of Texas M.D. Anderson Cancer Center Animal Care and Use Committee.

Western blot analysis

Primary MAFs were isolated from the peritoneal fascia of young adult mice as described (13) and were cultured in DMEM containing 10% fetal bovine serum (FBS). Primary thymocytes were isolated from 4- to 8-week-old mice and maintained in RPMI1640 medium containing 5% newborn calf serum. After treatment, cells were harvested at different time points and subjected to Western blot analysis for total p53 (Santa Cruz, p53-FL393, sc-6243), phospho-p53 Ser-15 (Cell Signaling, #9284), and p21 (Santa Cruz, sc-471). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH; Abcam, ab9485) and actin (Sigma) were used as loading controls. For mitochondrial localization experiments, primary murine embryonic fibroblasts (MEF) were subjected to fractionation using the ApoAlert Cell fractionation kit (Clontech). Hsp60 (Santa Cruz, sc-1722) was used as a mitochondrial marker; lamin A (Santa Cruz, sc-7293) was used as a control for nuclear contamination; and IκBα (Santa Cruz, sc-1643) was used as a control for cytoplasmic contamination. Cleaved poly ADP ribose polymerase (PARP; p85; Cell Signaling, #9542) was used as an indicator of apoptosis.

Quantitative real-time PCR analysis

Primary MEFs were isolated from E13.5 embryos and cultured in DMEM containing 10% FBS. Low-passage primary MEFs were exposed to 100 J/m^2 UVB, and total RNA was extracted using TRI Reagent (MRC). After reverse transcription, cDNAs were subjected to quantitative real-time PCR using Taqman Gene Expression Assays (Applied Biosystems). Genes that were analyzed include *p21, Puma, Perp*, and *Noxa*. *GAPDH* was used for normalization.

Immunohistochemical staining

Following UV or IR treatments, mice were euthanized, and UV-treated skin or IR-exposed intestine was removed and fixed in 10% neutral-buffered formalin. Formalin-fixed tissue was embedded in paraffin and sectioned for immunohistochemical staining to detect cells expressing p53 (Novocastra NCL-p53-CM5p), p21 (Santa Cruz, sc-6246), and cleaved caspase-3 (R&D Systems). Stained sections were examined microscopically and scored for the number of positive cells per 10 mm of linear epidermis or intestine.

UV skin carcinogenesis assay

Wild-type (WT; $p53^{+/+}$), $p53^{72R/72R}$, and $p53^{72P/72P}$ mice, backcrossed onto the SKH strain, were exposed to a daily dose of 300 J/m^2 of UVB light starting at 6 weeks of age. Each group consisted of 25 males and 25 females for a total of 50 mice per genotype. Mice were examined weekly, and the number of tumors per mouse was recorded. Tumors and skin samples were taken at the time of sacrifice and processed for histopathologic analysis. H&E-stained sections were examined by expert mouse pathologists for markers of malignancy, invasion, nuclear atypia, mitoses, acantholysis, subcutaneous fat, and inflammation.

Results

Generation of mouse models for the human R72P polymorphism

Because the R72P SNP lies within a region of *p53* that is not well conserved in the mouse, it was necessary to humanize the mouse to model this polymorphism (Supplementary Fig. S1). As the best way to model human genetic variation in the mouse was not clear, we chose two complementary strategies. One approach to humanize *p53* in the mouse was to generate transgenic lines using a BAC containing the entire human *p53* gene encoding either the p53R or p53P variant (Supplementary Fig. S2). The original BAC encoding p53R was modified by site-directed mutagenesis and homologous

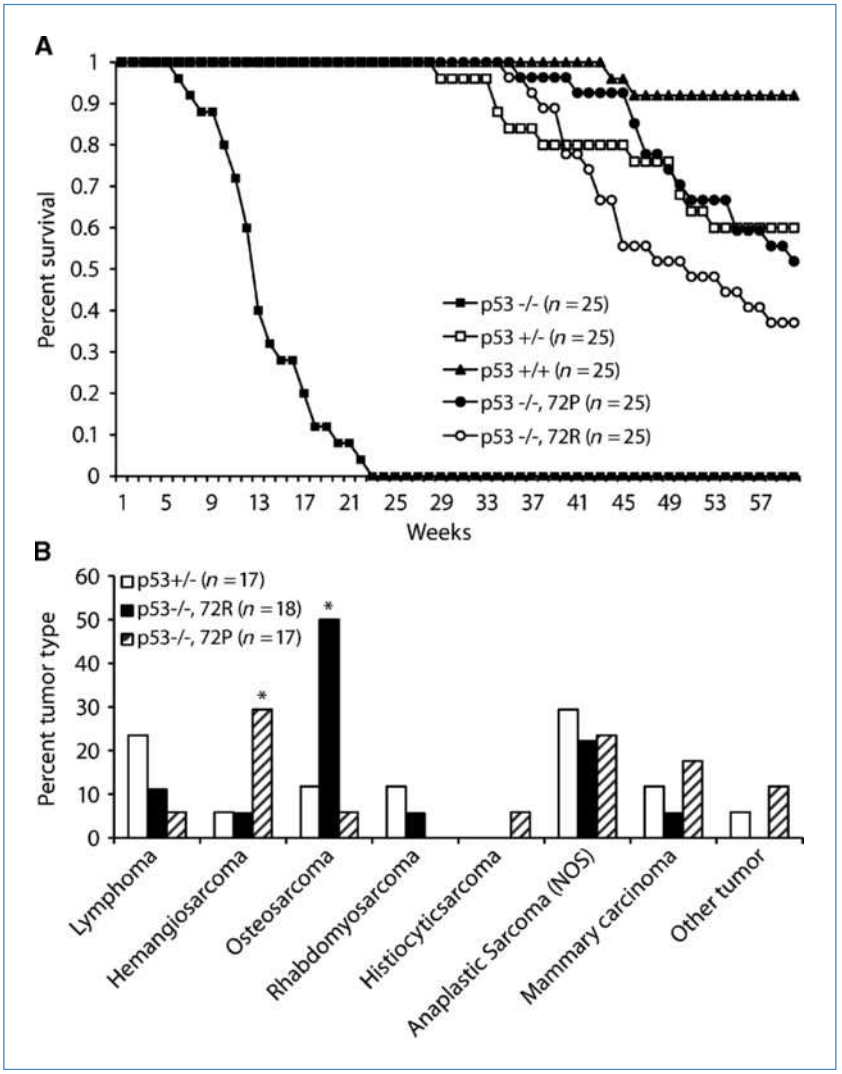

Figure 1. The human p53 BAC transgenes rescue $p53^{-/-}$ mice from early tumor development. A, BAC transgenic mice encoding either the human p53R or p53P variant in a $p53^{-/-}$ background (p53$^{-/-}$, 72R, and p53$^{-/-}$, 72P) together with $p53^{+/+}$, $p53^{+/-}$, and $p53^{-/-}$ sibling control mice were monitored for 60 wk for spontaneous tumor development. B, histopathologic analysis was performed on lesions from $p53^{+/-}$ mice and BAC transgenic mice in a $p53^{-/-}$ background. The percentage of tumor types in each category for each genotype is presented. *, statistically significant difference among genotypes (Fisher's exact test, $P < 0.05$).

recombination in bacteria to encode p53P. Transgenic lines containing a single copy of either the original or modified BAC were identified and crossed into a $p53^{-/-}$ background. Both BAC transgenes rescued $p53^{-/-}$ mice from early lymphoma development, indicating that each of the human p53 variants is functional for tumor suppression in the mouse (Fig. 1A). Interestingly, the human polymorphism seemed to alter the tumor type that developed in mice containing one WT or human *p53* allele. Osteosarcomas were more common in mice expressing p53R, whereas hemangiosarcomas were more common in mice expressing p53P compared with the other genotypes (Fig. 1B).

The other approach to model the R72P polymorphism used targeted homologous recombination in ES cells to replace mouse exon 4 with human exon 4 sequences, leaving the original surrounding mouse intronic sequences intact. This results in a chimeric p53 protein in which human amino acid sequences from 33 to 125 replace the analogous mouse sequences. A similar approach was used by others to generate the Hupki (human p53 knock-in) mouse model by knocking in exons 4 to 9 (14). The targeting constructs were based on existing constructs (9), and site-directed mutagenesis was used to introduce the SNP into human exon 4 (Supplementary Fig. S3). Correctly targeted ES cell clones containing human exon 4 in place of mouse exon 4 were identified for both polymorphic variants by Southern blot analysis and PCR (Supplementary Fig. S4). Established lines were crossed with CAG-Cre deleter mice to remove the puromycin selection cassette and allow expression of the humanized *p53* alleles in all tissues. Mice homozygous for either the codon 72R ($p53^{72R/72R}$) or codon 72P ($p53^{72P/72P}$) alleles were generated by interbreeding. These mice seem healthy and do not spontaneously develop early lymphomas or other tumors (data not shown).

Expression of p53 protein in the BAC transgenic and exon 4 knock-in models

Primary adult fibroblasts were isolated from the BAC transgenic mice backcrossed into a $p53^{-/-}$ background. Low levels of total p53 protein were observed in both BAC transgenic lines in the absence of DNA damage, similar to what was observed in WT mouse fibroblasts (Fig. 2A). Treatment with IR induced the expression of total p53 and phospho-serine 15 p53 in fibroblasts from both BAC transgenic lines to levels that were similar to what was observed in WT mouse fibroblasts (Fig. 2A). Similar results were obtained using primary fibroblasts isolated from the exon 4 knock-in models treated with IR (data not shown).

IR treatment of primary thymocytes isolated from $p53^{72R/72R}$ and $p53^{72P/72P}$ knock-in mice also resulted in p53 induction and phosphorylation (Fig. 2B). In this case, levels of both chimeric p53 protein variants seemed to be higher than mouse WT p53 at several time points. A similar trend of increased p53 protein levels in both knock-in models was also observed in primary MAFs exposed to UV radiation (Fig. 2C). Thus, in at least some cell types and conditions, the chimeric p53 protein variants may have increased stability compared with WT mouse p53 independent of the R72P polymorphism. Of note, both of the chimeric p53 variants seemed to be functional for the transcriptional induction of p21 (Fig. 2B and C).

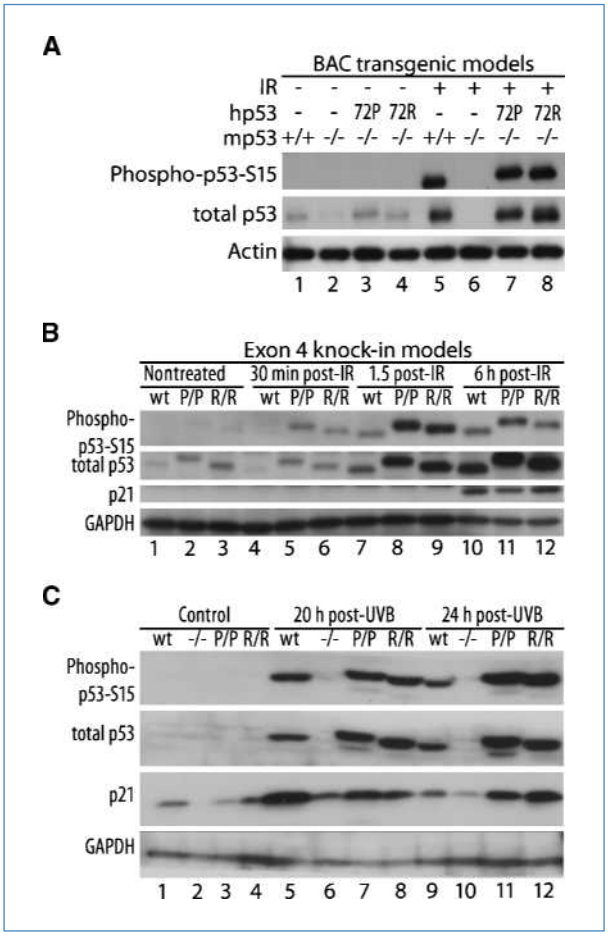

Figure 2. Induction of p53 in cells from the BAC transgenic and exon 4 knock-in mice in response to DNA-damaging agents. A, primary MAFs were isolated from BAC transgenic mice encoding the human p53 (hp53) variants (72P, lanes 3 and 7, or 72R, lanes 4 and 8) in a mouse $p53^{-/-}$ (mp53, –/–) background along with WT (+/+; lanes 1 and 5) and $p53^{-/-}$ (lanes 2 and 6) control mice. These MAFs were nontreated (lanes 1–4) or exposed to 6 Gy of IR (lanes 5–8) and harvested at 1.5 h postexposure. Cell lysate was used to examine total and phospho-p53 (S15) by Western blot analysis as indicated. B, thymocytes were isolated from WT (wt; lanes 1, 4, 7, and 10) or the human exon 4 knock-in mice (P/P, lanes 2, 5, 8, and 11, or R/R, lanes 3, 6, 9, and 12) and left nontreated (lanes 1–3) or exposed to 6 Gy of IR (lanes 4–12). Cells were harvested at various time points after IR treatment and subjected to Western blot analysis to examine total and phospho-p53 (S15), p21, and GAPDH. C, primary MAFs were isolated from WT (wt; lanes 1, 5, and 9), $p53^{-/-}$ (lanes 2, 6, and 10), and exon 4 knock-in mice (P/P, lanes 3, 7, and 11, or R/R, lanes 4, 8, and 12). MAFs were nontreated (lanes 1–4) or exposed to UVB radiation (500 J/m^2) and harvested 20 (lanes 5–8) or 24 h (lanes 9–12) postexposure. Western blot analysis was performed to examine total and phospho-p53 (S15), p21, and GAPDH.

Increased apoptotic capacity of the p53R variant compared with p53P in the mouse models

To examine the response to UV radiation *in vivo*, exon 4 knock-in mice were exposed to UVB and skin samples taken

24 hours postirradiation. The number of epidermal keratinocytes staining positive for p53 and the transcriptional target p21 were similar among $p53^{+/+}$, $p53^{72P/72P}$, and $p53^{72R/72R}$ mice (Supplementary Figs. S5 and S6; Fig. 3A). Similar results were obtained using the BAC transgenic models expressing the human p53 variants in a $p53^{-/-}$ background except that the number of p21-positive cells was higher in UV-treated WT mice compared with either BAC transgenic mouse line (Supplementary Fig. S5; Fig. 3).

The apoptotic response to UV was examined in these skin samples by staining for the activated (cleaved) form of caspase-3. Interestingly, $p53^{72R/72R}$ mice showed significantly increased levels of apoptotic cells following UV irradiation compared with $p53^{+/+}$ and $p53^{72P/72P}$ mice (Fig. 3B). Similar results were obtained by counting morphologically distinct "sunburn" cells in H&E-stained skin sections (Supplementary Fig. S6D). As with the knock-in models, BAC transgenic mice expressing the p53R variant also showed an increased apoptotic response to UV compared with WT and BAC transgenic mice expressing the p53P variant (Fig. 3D). Thus, in humanized mouse models developed using two different approaches, the p53R variant displays greater apoptotic activity after UV exposure, consistent with *in vitro* studies using human cells.

The knock-in mouse models were also used to examine the apoptotic response to IR in the intestine. In contrast to nontreated control samples, many cells stained positive for p53 and p21 at 1.5 hours post-IR treatment, and this response was similar among $p53^{+/+}$, $p53^{72P/72P}$, and $p53^{72R/72R}$ mice (Supplementary Fig. S7; Fig. 4A and B). However, at the later 24-hour post-IR time point, there were fewer p53-positive cells in WT intestines compared with intestines from $p53^{72P/72P}$ and $p53^{72R/72R}$ mice (Fig. 4A). As mentioned, this may indicate increased stability of the chimeric p53 proteins compared with WT mouse p53.

Similar to their response to UV, $p53^{72R/72R}$ mice displayed higher numbers of apoptotic cells in the intestine in response to IR compared with $p53^{72P/72P}$ mice (Fig. 4C). This was particularly evident at the early 1.5-hour post-IR time point. However, in the case of IR-exposed intestine, $p53^{72R/72R}$ and WT mice showed a similar apoptotic response, whereas $p53^{72P/72P}$ mice had a lower apoptotic response. Thus, although the same functional difference in apoptotic capacities is observed in the different tissues, the p53R variant seems

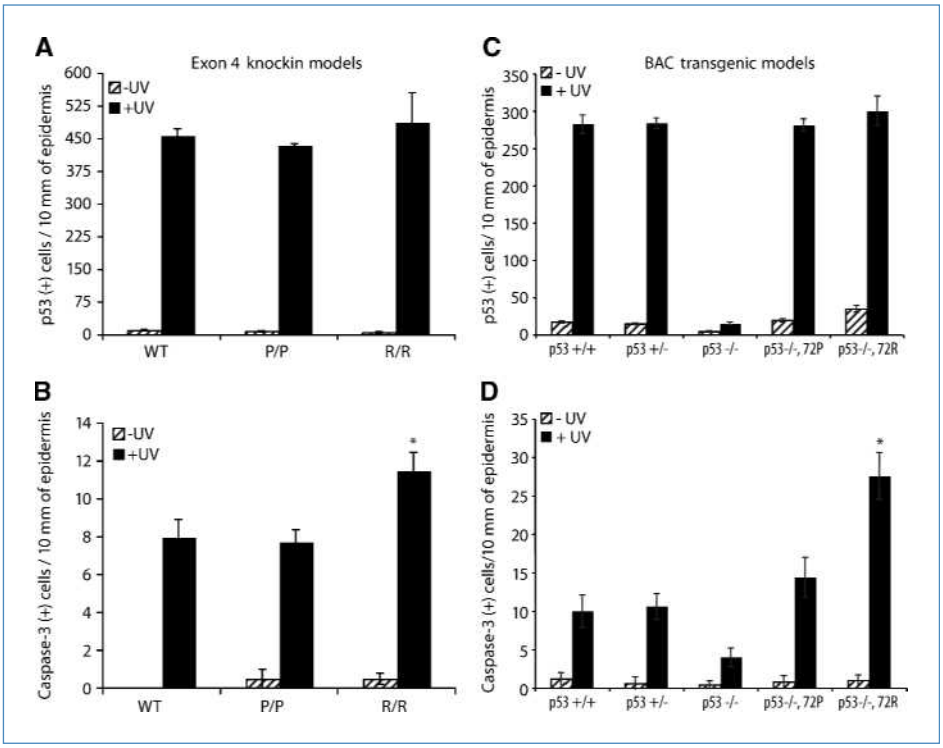

Figure 3. Increased sensitivity of mice expressing the p53R variant to UVB-induced apoptosis. A and B, 6- to 8-wk-old WT (+/+), $p53^{72P/72P}$ (P/P), and $p53^{72R/72R}$ (R/R) mice were irradiated with 300 J/m^2 of UVB. Mice were sacrificed 24 h posttreatment, and the dorsal skin was taken as the UV-treated samples whereas the ventral skin was used as unexposed control samples. Skin sections were immunostained for (A) p53 or (B) the activated (cleaved) form of caspase-3. The average number of positive cells per 10 mm of epidermis is presented from four mice per genotype. C and D, mice WT (+/+), hemizygous (+/–), or nullizygous (–/–) for mouse *p53* along with humanized BAC transgenic mice encoding either p53P (72P) or p53R (72R) were exposed to 1,000 J/m^2 of UVB. Twenty-four hours after exposure, skin samples were taken and immunostained for (C) total p53 or (D) activated caspase-3. The average number of positive cells per 10 mm of linear epidermis was determined microscopically from five mice per genotype. *, significant difference from other groups treated similarly according to the Student's *t* test, $P < 0.05$.

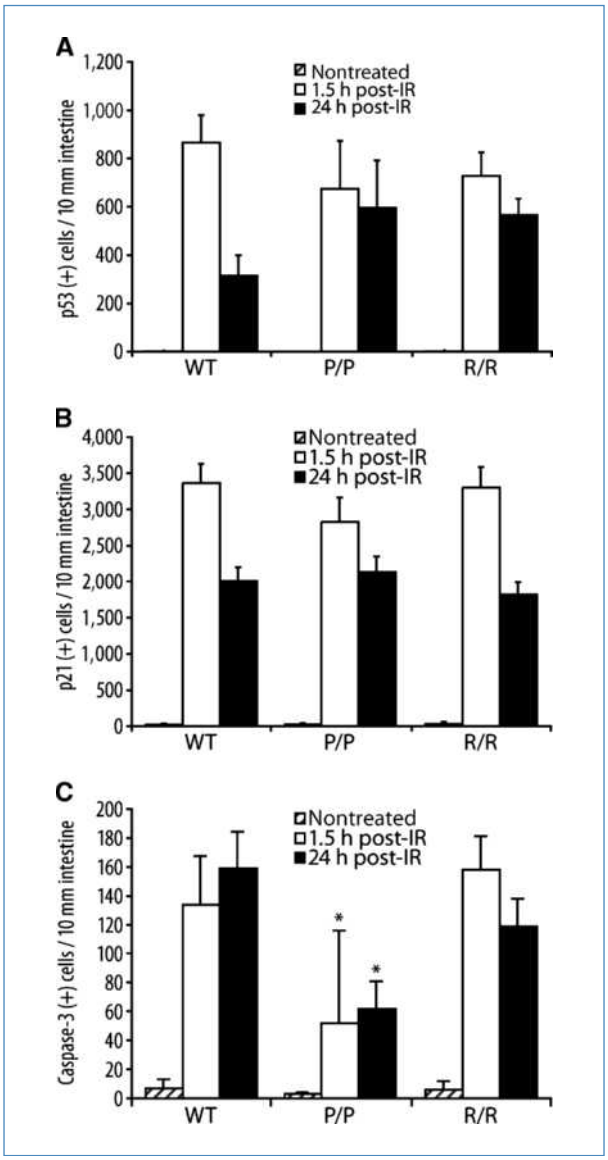

Figure 4. Increased sensitivity of $p53^{72R/72R}$ mice to IR-induced apoptosis. Young adult WT, $p53^{72P/72P}$ (P/P), and $p53^{72R/72R}$ (R/R) mice were exposed to 6 Gy of IR and sacrificed 1.5 or 24 h postirradiation. Intestine samples were taken and immunostained for (A) total p53, (B) p21, or (C) activated caspase-3. Stained sections were examined microscopically, and the average number of positive cells per 10 mm of linear intestine was determined from three mice per genotype. *, statistically significant differences from other groups treated similarly according to the Student's *t* test, $P < 0.05$.

to be hyperactive in UV-treated skin, whereas p53P is hypoactive in IR-treated intestine compared with WT mouse p53.

The R72P polymorphism affects the induction of proapoptotic genes and mitochondrial localization in mouse models

It has been suggested that the increased apoptotic activity of the p53R variant is due to its increased ability to transcriptionally activate certain proapoptotic genes (5). To test this hypothesis in our mouse models, quantitative real-time PCR was used to examine the expression of selected p53 target genes in UV-irradiated primary fibroblasts isolated from the knock-in mouse models. The induction of *p21, Perp, Puma*, and *Noxa* in response to UV treatment was p53 dependent as shown by the lack of induction in $p53^{-/-}$ cells. The expression of *p21* increased to similar levels in WT, $p53^{72P/72P}$, and $p53^{72R/72R}$ cells (Fig. 5A). In contrast, a significant difference in the expression of *Perp, Puma*, and *Noxa* was observed between $p53^{72P/72P}$ and $p53^{72R/72R}$ cells exposed to UV, with *Perp* also induced to significantly higher levels in $p53^{72R/72R}$ cells compared with WT cells (Fig. 5B–D). It should be noted that the basal levels of expression for these three proapoptotic genes was also significantly higher in $p53^{72R/72R}$ cells compared with WT or $p53^{72P/72P}$ cells.

Another mechanism that has been proposed for the difference in apoptotic capacities between the polymorphic variants involves a differential ability to localize to mitochondria (6, 7). Previous studies have shown that p53 can induce apoptosis independent of transcriptional regulation by interacting directly with Bcl2 family members at the mitochondrial membrane (15, 16). Although no difference in the abilities of the variants to interact with Bcl2 family members was observed *in vitro*, p53R was shown to exit the nucleus and localize to the mitochondria more efficiently than p53P (7).

Consistent with previous results, UV irradiation induced similar levels of p53 protein in primary fibroblasts isolated from $p53^{72P/72P}$ and $p53^{72R/72R}$ mice as measured by Western blot analysis of whole-cell extracts (Fig. 6A). However, a higher level of the p53R variant protein was found associated with mitochondria compared with the p53P variant protein. As previously observed, the humanized p53 chimeric proteins were induced to higher levels compared with WT mouse p53. Thus, it is difficult to compare the mitochondrial localization efficiency of WT mouse p53 to that of the humanized p53 proteins.

Similar results were obtained in response to the DNA-damaging drug camptothecin. Drug treatment induced similar levels of p53 protein in primary fibroblasts isolated from $p53^{72P/72P}$ and $p53^{72R/72R}$ mice as measured by Western blot analysis of whole-cell extracts (Fig. 6B). However, an increased level of p53 protein was found in mitochondrial fractions isolated from $p53^{72R/72R}$ cells following treatment. Consistent with increased localization of p53R to mitochondria, $p53^{72R/72R}$ cells also displayed increased levels of the p85 fragment of PARP, a marker of apoptosis. Taken together, these findings suggest that both transcriptional and nontranscriptional mechanisms may contribute to the higher apoptotic activity of the p53R polymorphic variant.

The R72P polymorphism does not significantly affect UV-induced carcinogenesis

The p53-dependent apoptotic response to UV radiation is thought to play an important role in suppressing skin tumor development (17). Indeed, previous studies have shown that deletion or mutation of *p53* in mice significantly

enhances skin tumor development in response to chronic UV exposure (12, 18, 19). Reports have also suggested that the R72P polymorphism modulates the risk for developing skin cancer as a result of the differential apoptotic response to sunlight (20, 21).

To determine if differences in the acute response to UV would translate into differences in tumor susceptibility, $p53^{72P/72P}$, $p53^{72R/72R}$, and WT control mice were exposed to a daily dose of UVB starting at 6 weeks of age. Surprisingly, no statistically significant difference in latency time or tumor multiplicity was observed among the genotypes (Supplementary Fig. S8A and B). Histopathologic analysis of the skin tumors also found no significant differences in degree of malignancy, invasion, or inflammation between the three genotypes (data not shown).

Discussion

Mouse models have been instrumental in revising our understanding of p53 regulation and function, and have shown the importance of studying p53 in an *in vivo* system (22). Weinberg and colleagues (23, 24) have argued for the need to more accurately model human disease in the mouse and suggest that one way to do this is to generate humanized mouse models in which functionally important human sequences are exchanged for the mouse orthologues. Here, we have described the generation and first analyses of humanized mouse models for the study of the naturally occurring and functionally relevant R72P polymorphism of p53. A caveat of these models is whether human p53 or chimeric p53 proteins would function normally in the mouse. Two groups have previously generated mouse models humanized for the p53R polymorphic variant using approaches similar to the ones used here (14, 25). A transgenic model containing multiple copies of a 20-Kb human genomic fragment in a $p53^{-/-}$ background was found to express normal levels of human p53 protein and was found to be rescued from early lymphomagenesis but was defective for p53-dependent transcriptional activation and apoptosis (25). On the other hand, the Hupki model in which human exons replace mouse exons 4 to 9 displays apparently normal p53 function (14), similar to the models described here.

Previous studies showed that the R72P polymorphism affects the apoptotic capacity of p53, with the p53R variant being a more potent inducer of apoptosis than the p53P variant. However, these studies were all performed using rather artificial *in vitro* systems that could adversely affect the analysis of p53 functioning. The mouse models described here allow for the study of this polymorphism under experimentally defined and physiologically relevant conditions. Importantly, we find that the effect of the R72P polymorphism on the apoptotic activity of p53 is recapitulated in the BAC transgenic and exon 4 knock-in models. These findings contradict a recent report suggesting the differential effects of the human R72P polymorphism are absent in mouse cells (26). The reason for this discrepancy is unclear but may be

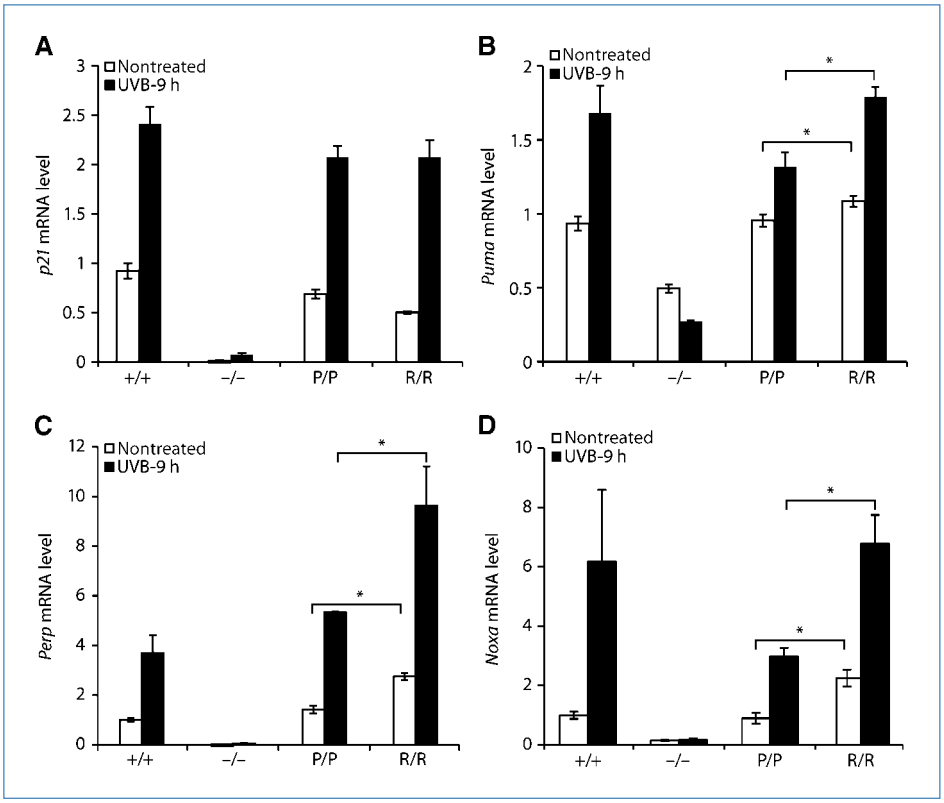

Figure 5. The R72P polymorphism affects the expression of proapoptotic genes in the knock-in mouse models. Primary MEFs isolated from WT (+/+), $p53^{-/-}$ (−/−), $p53^{72P/72P}$ (P/P), and $p53^{72R/72R}$ (R/R) embryos were nontreated or treated with 100 J/m^2 of UVB. Total RNA was extracted 9 h after treatment and subjected to quantitative real-time PCR to examine mRNA levels of (A) *p21*, (B) *Puma*, (C) *Perp*, and (D) *Noxa*. The average from triplicate plates of cells is presented. *, statistical significance between $p53^{72P/72P}$ and $p53^{72R/72R}$ genotype by Student's *t* test ($P < 0.05$).

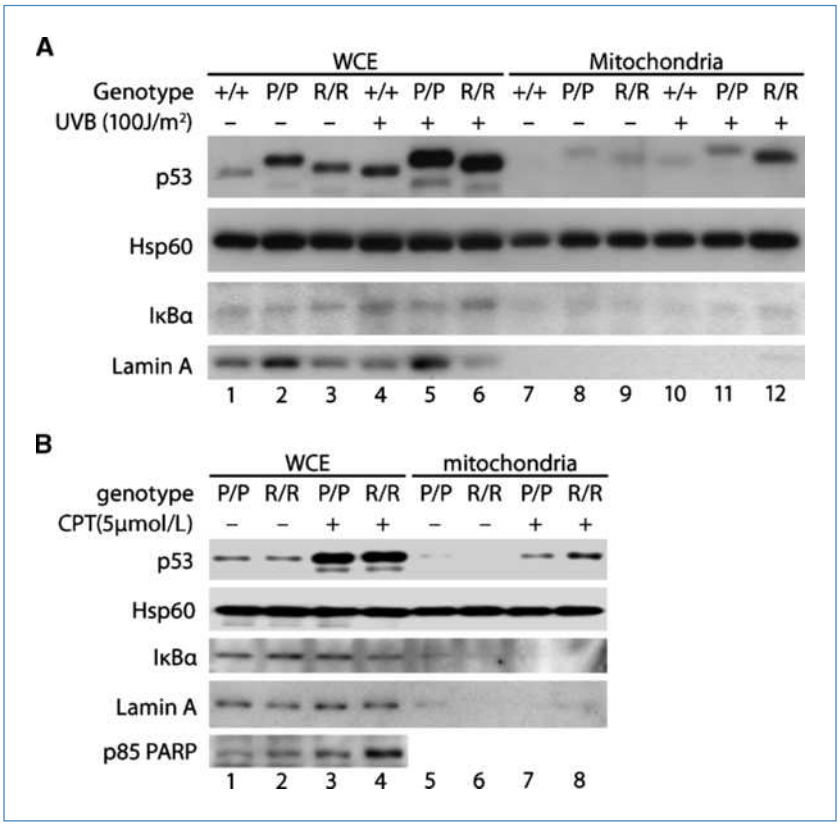

Figure 6. The R72P polymorphism affects p53 mitochondrial localization in the knock-in mouse models. A, primary MEFs isolated from WT (+/+; lanes 1, 4, 7, and 10), $p53^{72P/72P}$ (P/P; lanes 2, 5, 8, and 11), and $p53^{72R/72R}$ (R/R; lanes 3, 6, 9, and 12) mice were nontreated (lanes 1–3 and 7–9) or irradiated with 100 J/m^2 of UVB (lanes 4–6 and 10–12). Western blot analysis was performed on whole-cell extracts (lanes 1–6) or isolated mitochondrial extracts (lanes 7–12) using antibodies to total p53, HSP60 (as a mitochondrial marker), IκBα (as control for cytoplasmic contamination), and lamin A (as control for nuclear contamination). B, primary MEFs isolated from $p53^{72P/72P}$ (lanes 1, 3, 5, and 7) or $p53^{72R/72R}$ (lanes 2, 4, 6, and 8) mice were nontreated (lanes 1, 2, 5, and 6) or treated with 5 μmol/L of camptothecin (CPT) for 5 h before harvesting. Western blot analysis was performed on whole-cell extracts (lanes 1–4) or isolated mitochondrial extracts (lanes 5–8) using antibodies to total p53, HSP60, IκBα, lamin A, and the p85 fragment of PARP as an indicator of apoptosis.

related to the artificial conditions, including overexpression, used in that *in vitro* study.

It should be noted that during the review of this article, two reports were published describing mouse models of human polymorphisms in the *FGFR4* and the *BDNF* genes (27, 28). Mouse models for a functional polymorphism in the *MDM2* gene have also recently been developed.[6] Taken together, these models establish that the mouse can be used as a tool to study human genetic variation.

Two distinct, but not mutually exclusive, mechanisms have been suggested to underlie the difference in apoptotic potential associated with the R72P polymorphism. One mechanism involves a differential ability to transcriptionally activate certain proapoptotic target genes. Similar to what has been observed in engineered human cell lines (5), we find that the p53 polymorphic variants induce similar levels of *p21* but that p53R is a stronger activator of *Puma, Perp*, and *Noxa* compared with p53P. The other mechanism suggested to be responsible for the difference in apoptotic potential involves effects of the R72P polymorphism on nuclear export and mitochondrial localization (6, 7). Consistent with those studies, we find that p53R localizes to mitochondrial fractions more efficiently than p53P in cells from the knock-in mouse models. Thus, both transcriptional and nontranscriptional mechanisms may contribute to the higher apoptotic capacity of p53R in the mouse models.

Although $p53^{72R/72R}$ knock-in mice had an increased acute apoptotic response to UV radiation, they were as susceptible to tumor development as WT and $p53^{72P/72P}$ mice in a chronic UV carcinogenicity assay. One explanation for this could be that the polymorphism results in a subtle phenotype that is concealed by chronic exposure to relatively high doses of UV. More accurate modeling to mimic human exposure may be required to observe differences in skin tumor susceptibility. Alternatively, these results could indicate that the acute apoptotic response to UV is not the primary or sole mechanism by which p53 suppresses skin tumor development. Indeed, Evan and coworkers (29) recently used an inducible p53 mouse model system to show that the acute response to IR was irrelevant for suppressing lymphomagenesis.

It has also been shown that the R72P polymorphism affects the gain-of-function activity of mutant p53. In particular, mutant forms of p53R are more efficient than analogous mutant forms of p53P in binding to p73, suppressing p73-mediated apoptosis, and contributing to oncogenic transformation (30). Given that p53 mutations are an early event in UV carcinogenesis, it may be that the gain-of-function activity of mutant p53R offsets the protective effect of enhanced apoptosis, leading to no overall change in tumorigenesis. Future studies will examine how the R72P SNP modulates the activity of mutant p53

[6] Sean Post and Guillermina Lozano, personal communication.

in the mouse models and how this affects tumorigenesis and the response to therapies.

In addition to skin cancer, the R72P polymorphism has also been shown to modify the risk for developing other cancers, including lung, breast, colon, and prostate cancers (2). Most studies find that the presence of one or two p53P-encoding alleles is associated with an increased risk of developing cancer, consistent with the decreased apoptotic activity of p53P. However, the R/R genotype has been reported to increase the risk for some cancers, particular those associated with human papilloma virus (HPV) infections. This may be due to an enhanced ability of the HPV E6 oncoprotein to target p53R for degradation compared with p53P (3). The R72P mouse models described here should be useful for studying the role of this polymorphism in modulating cancer development in different tissues and in response to different environmental insults, such as HPV infection. Moreover, these models may also be useful for studying the potential role of the R72P SNP in other diseases and in aging (31).

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank Edwin Zwart, Aart Verhoef, Jennifer Smith, Pam Blau, Stephanie Clifford, John Repass, and Karla Fuller for technical assistance; Kevin Lin for the statistical analysis; Lezlee Coghlan, Dale Weiss, and coworkers for the animal care; Christine Brown and Wendy Rodenburg for the assistance with figures; Dr. Guillermina Lozano for the helpful comments, and Shawnda Sanders and Becky Brooks for the manuscript preparation.

Grant Support

These mouse models were developed as part of the Comparative Mouse Genomics Centers Consortium (U01 ES011047 and U01 ES11044) with additional support from NIH grants ES015587 (D.G. Johnson), P30ES007784, CA016672, P50CA097007, and training grant NIH CA009480 (K. Fuller).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received 12/21/2009; revised 04/29/2010; accepted 05/16/2010; published OnlineFirst 06/29/2010.

References

1. Available from: http:/snp500cancer.nci.nih.gov.
2. Whibley C, Pharoah PD, Hollstein M. p53 polymorphisms: cancer implications. Nat Rev Cancer 2009;9:95–107.
3. Thomas M, Kalita A, Labrecque S, et al. Two polymorphic variants of wild-type p53 differ biochemically and biologically. Mol Cell Biol 1999;19:1092–100.
4. Bergamaschi D, Samuels Y, Sullivan A, et al. iASPP preferentially binds p53 proline-rich region and modulates apoptotic function of codon 72-polymorphic p53. Nat Genet 2006;38:1133–41.
5. Sullivan A, Syed N, Gasco M, et al. Polymorphism in wild-type p53 modulates response to chemotherapy *in vitro* and *in vivo*. Oncogene 2004;23:3328–37.
6. Dumont P, Leu JI, Della Pietra AC III, George DL, Murphy M. The codon 72 polymorphic variants of p53 have markedly different apoptotic potential. Nat Genet 2003;33:357–65.
7. Leu JI, Dumont P, Hafey M, Murphy ME, George DL. Mitochondrial p53 activates Bak and causes disruption of a Bak-Mcl1 complex. Nat Cell Biol 2004;6:443–50.
8. Lalioti M, Heath J. A new method for generating point mutations in bacterial artificial chromosomes by homologous recombination in Escherichia coli. Nucleic Acids Res 2001;29:E14.
9. de Vries A, Flores ER, Miranda B, et al. Targeted point mutations of p53 lead to dominant-negative inhibition of wild-type p53 function. Proc Natl Acad Sci U S A 2002;99:2948–53.
10. Olive KP, Tuveson DA, Ruhe ZC, et al. Mutant p53 gain of function in two mouse models of Li-Fraumeni syndrome. Cell 2004;119:847–60.
11. Berton TR, Mitchell DL, Guo R, Johnson DG. Regulation of epidermal apoptosis and DNA repair by E2F1 in response to ultraviolet B radiation. Oncogene 2005;24:2449–60.
12. Bruins W, Zwart E, Attardi LD, et al. Increased sensitivity to UV radiation in mice with a p53 point mutation at Ser389. Mol Cell Biol 2004;24:8884–94.
13. Powers JT, Hong S, Mayhew CN, et al. E2F1 uses the ATM signaling pathway to induce p53 and Chk2 phosphorylation and apoptosis. Mol Cancer Res 2004;2:203–14.
14. Luo JL, Yang Q, Tong WM, et al. Knock-in mice with a chimeric human/murine p53 gene develop normally and show wild-type p53 responses to DNA damaging agents: a new biomedical research tool. Oncogene 2001;20:320–8.
15. Chipuk JE, Kuwana T, Bouchier-Hayes L, et al. Direct activation of Bax by p53 mediates mitochondrial membrane permeabilization and apoptosis. Science 2004;303:1010–4.
16. Mihara M, Erster S, Zaika A, et al. p53 has a direct apoptogenic role at the mitochondria. Mol Cell 2003;11:577–90.
17. Ziegler A, Jonason AS, Leffell DJ, et al. Sunburn and p53 in the onset of skin cancer [see comments]. Nature 1994;372:773–6.
18. Jiang W, Ananthaswamy HN, Muller HK, Kripke ML. p53 protects against skin cancer induction by UV-B radiation. Oncogene 1999;18:4247–53.
19. Wijnhoven SW, Speksnijder EN, Liu X, et al. Dominant-negative but not gain-of-function effects of a p53.R270H mutation in mouse epithelium tissue after DNA damage. Cancer Res 2007;67:4648–56.
20. Han J, Cox DG, Colditz GA, Hunter DJ. The p53 codon 72 polymorphism, sunburns, and risk of skin cancer in US Caucasian women. Mol Carcinog 2006;45:694–700.
21. McGregor JM, Harwood CA, Brooks L, et al. Relationship between p53 codon 72 polymorphism and susceptibility to sunburn and skin cancer. J Invest Dermatol 2002;119:84–90.
22. Wahl GM. Mouse bites dogma: how mouse models are changing our views of how P53 is regulated *in vivo*. Cell Death Differ 2006;13:973–83.
23. Rangarajan A, Weinberg RA. Opinion: Comparative biology of mouse versus human cells: modelling human cancer in mice. Nat Rev Cancer 2003;3:952–9.
24. Hahn WC, Weinberg RA. Modelling the molecular circuitry of cancer. Nat Rev Cancer 2002;2:331–41.
25. Dudgeon C, Kek C, Demidov ON, et al. Tumor susceptibility and apoptosis defect in a mouse strain expressing a human p53 transgene. Cancer Res 2006;66:2928–36.
26. Phang BH, Sabapathy K. The codon 72 polymorphism-specific effects of human p53 are absent in mouse cells: implications on generation of mouse models. Oncogene 2007;26:2964–74.
27. Seitzer N, Mayr T, Streit S, Ullrich A. A single nucleotide change in the mouse genome accelerates breast cancer progression. Cancer Res 2010;70:802–12.
28. Soliman F, Glatt CE, Bath KG, et al. A genetic variant BDNF polymorphism alters extinction learning in both mouse and human. Science 2010;327:863–6.
29. Christophorou MA, Ringshausen I, Finch AJ, Swigart LB, Evan GI. The pathological response to DNA damage does not contribute to p53-mediated tumour suppression. Nature 2006;443:214–7.
30. Marin MC, Jost CA, Brooks LA, et al. A common polymorphism acts as an intragenic modifier of mutant p53 behaviour. Nat Genet 2000;25:47–54.
31. van Heemst D, Mooijaart SP, Beekman M, et al. Variation in the human TP53 gene affects old age survival and cancer mortality. Exp Gerontol 2005;40:11–5.