p53 Transgenic Mice Are Highly Susceptible to 1, 2-Dimethylhydrazine-induced Uterine Sarcomas

Zhongju Zhang, Jie Li, Laura E. Lantry, Yian Wang, Roger W. Wiseman, Ronald A. Lubet, and Ming You

Division of Human Cancer Genetics (Z. Z., J. L. L., M. Y.) and School of Public Health (Y. W.), The Ohio State University Comprehensive Cancer Center, Columbus, Ohio 43210; Department of Pathology, Medical College of Ohio, Toledo, Ohio 43699; Laboratory of Women’s Health, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709; and Chemoprevention Branch, National Cancer Institute, Bethesda, Maryland 20892

ABSTRACT

p53 Transgenic mice were crossed with C57BL/6J mice to investigate whether a germ-line mutation in the p53 gene predisposes for tumorigenesis in mice. (C57BL/6J × UL53–3) F1 mice were treated with 1,2-dimethylhydrazine (DMH), a colon carcinogen. The presence of a mutant p53 led to an increased incidence or multiplicity of uterine sarcomas, colon carcinomas, lung adenomas, and hepatomas in DMH-treated mice. The most significant effect of mutant p53 was the increased incidence of uterine sarcomas, which were found in ~90% of p53val135/WT mice but only seen in ~10% of p53WT/WT mice. After examination of 15 known p53 downstream target genes in uterine sarcomas and normal uteri, we found that expression of the Reprimo gene was significantly increased in normal uteri of p53WT/WT but not in either normal uterus or uterine sarcomas of p53val135/WT mice. In DMH-treated animals, long-term treatment with this chemopreventive agent, piroxicam, reduced colon cancer incidence and multiplicity in both p53val135/WT or p53WT/WT mice but did not affect the formation of uterine sarcomas, lung adenomas, or hepatomas. These results demonstrate a tissue-specific enhancement of tumorigenesis in multiple organs by the mutant p53 transgene and additionally support the utility of (C57BL/6J × UL53–3) F1 mice for chemoprevention studies.

INTRODUCTION

The p53 gene is the most frequently mutated gene in human cancer (1). Half or more of human tumors carry mutations in this gene, which is thought to act as a tumor suppressor by regulating the cell cycle and apoptosis (2, 3). Inheritance of a mutant p53 allele is a hallmark of LFS, a rare autosomal-dominant disease that predisposes family members to many cancers (4–7). Having a mutant p53 allele results in a 25-fold increase in the likelihood of developing cancer by 50 years of age (8). Several studies demonstrate that mutant p53 has a gain-of-function effect and/or a dominant negative effect on the wild-type p53 protein in LFS cells (9, 10). Lymphoblastoid cell lines or peripheral lymphocytes derived from individuals with LFS exhibit an abnormal response to DNA damage and abnormal apoptotic activities after exposure to γ-irradiation. Presumably this is because mutant p53 cannot arrest cells at G1 to permit DNA repair or apoptosis (11, 12).

The p53 gene is a tumor suppressor gene that can integrate signals from multiple pathways to control the cell cycle and apoptosis (13). p53 can be activated by DNA damage (ATM and Chk2), aberrant growth signals (p14ARF), and chemotherapeutic drugs/UV light/protein-kinase inhibitors (14–17). After p53 is activated, it can bind to regulatory DNA sequences and activate the expression of p53 target genes, which ultimately leads to growth arrest, apoptosis, and inhibition of angiogenesis (13). p53 regulates genes that can be grouped into four categories: cell cycle inhibition (p21, GADD45, 14–3-3, and Reprimo), apoptosis (Scotin, PERP, NOXA, KILLER/DR5, p53AIP1, Fas, Bax, and PIDD), genetic stability, and inhibition of angiogenesis (TSP1, Maspin, BA1, and GD-AIF; Refs. 13–17). Inactivating p53 mutations in patients with LFS and in a variety of sporadic tumors reduce the function of p53 protein, which leads to an inability to activate p53 target genes. Analyzing the expression of p53 target genes permits a determination of how a mutant p53 contributes to tumor development in specific cell types or tissues.

Development of mouse tumor models carrying germ-line mutations in the p53 gene can greatly facilitate analysis of the role of p53 in tumorigenesis and simultaneously offer relevant in situ preclinical models for studies of cancer chemoprevention and therapy. Initial studies used animals with a knockout of one or both copies of p53 (18–21). These animals are more susceptible to a variety of carcinogens and have proven useful in a variety of prevention and therapeutic studies (18–24). For example, Hursting et al. (18, 21) have shown that caloric restriction and chemopreventive steroids can suppress spontaneous tumor development in p53 knockout mice. p53 knockout mice have been used in evaluating mechanisms underlying some of the efficacious interventions (25, 26). Nevertheless, p53 knockout mice have limitations. First, knockout of the p53 gene as a primary mechanism for altering p53 in humans is quite rare (1). More typically, this is accomplished by a mutation, often a dominant negative mutation, followed in some cases by loss of the corresponding “normal” p53 allele (1). Second, primary spontaneous tumors in p53 knockout mice are predominately lymphomas and sarcomas. To generate a model that more closely resembles cancer types seen in humans, a p53 genomic clone containing an Ala 135 Val mutation was microinjected into fertilized eggs of FVB mice (27). The resulting transgenic mice, carrying a missense p53 mutation (Ala135Val), developed a high occurrence of lung adenocarcinomas, osteosarcomas, lymphomas, and soft tissue sarcomas (27). The primary goal of the present study was to examine the effect of a p53 mutant transgene on tumorigenesis in mice treated with DMH, a colon carcinogen.

Multiple studies in mice and rats have shown that piroxicam and other NSAIDs inhibit colon carcinogenesis. The inhibitory effects appear to be mediated by the ability of NSAIDs to inhibit cyclooxygenase-1 on COX-1 and COX-2 enzymes, and to enhance apoptosis in colon tumor cells or carcinogen-initiated cells (28–32). Therefore, we examined the efficacy of piroxicam, a nonspecific NSAID that affects both COX-1 and COX-2 activities, on DMH-induced tumorigenesis in both p53val135/WT or p53WT/WT mice. Piroxicam was found to be effective in the presence or absence of a mutated p53 transgene.

MATERIALS AND METHODS

Reagents. DMH (99% pure) and piroxicam (99% pure) were purchased from Sigma Chemical Co. (St. Louis, MO).

Animals. UL53–3 mice carrying a 135Val p53 mutation in exon 5 were obtained from the National Institute of Environmental Health Sciences (Research Triangle Park, NC). C57BL/6J mice were obtained from The Jackson Laboratory. DMH and piroxicam were prepared in 5% methylcellulose, injected subcutaneously (20 mg/kg and 10 mg/kg, respectively) once daily for 14 days. For pregnancy studies involving DMH treatment, mice were administered two injections per day on day 7 and day 12 of gestation. For chemoprevention studies involving piroxicam treatment, mice were administered 20 mg/kg and 10 mg/kg per day for 14 days. Mice were sacrificed by CO2 asphyxiation and tissue was collected for histology or DNA isolation.

Histology. Mouse tissues were fixed in 10% formalin, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin. Uterus, colon, lungs, and skin were examined for the presence of lesions.

DNA Microarray Analysis. DNA was extracted from p53wt/wt and p53val135/wt uteri and microarray analysis was performed as previously described.

Received 8/29/01; accepted 4/4/02.

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.

1 These authors contributed equally to this work.

2 Two to three mice used for the 32P-orthophosphate pulse chase experiment were used.

3 Supported in part by USPHS Grants CN05122, CA58554, CA78797, and CA16058.

4 The abbreviations used are: LFS, Li-Fraumeni syndrome; DMH, 1,2-dimethylhydrazine; OH-BBN, N-butyl-N-(4-hydroxybutyl)-nitrosamine; NSAID, nonsteroidal anti-inflammatory drug; UL53–3 mice, mice containing mutant p53 transgene (135val) wt, wild-type; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; RT-PCR, reverse transcription-PCR.
Laboratory (Bar Harbor, ME). All of the mice used in this study were (C57BL/6J × UL53–3) F1 mice. The reason for crossing the transgene onto a C57BL/6J background was to determine the effect of the p53 transgene on colon carcinogenesis because the C57BL/6J mouse is relatively more susceptible to colon carcinogenesis than FVB/J background. Mice were housed 4 per cage, in plastic cages with hardwood bedding and dust covers, in a HEPA filtered, environmentally controlled (24 °C, 12 h light/dark cycle) room. Animals for the DMH carcinogenesis study were fed AIN-76A Purified Diet #100000 (Dyets Inc., Bethlehem, PA).

**p53 Genotype.** UL53–3 mice were developed by microinjection of FVB/J mouse oocytes with a BALB/c mouse genomic clone of the p53 gene containing a point mutation at codon 135 (Ala → Val) in exon 5 (27). The mutation, a C → T transition, created a restriction fragment length polymorphism with a new HhaI restriction enzyme cleavage site (recognition site: GGTGA). This mutation was used to genotype (C57BL/6J × UL53–3) F1 mice, using the PCR-restriction fragment length polymorphism method as described previously (33).

**Treatment with DMH.** At 6 weeks of age, (C57BL/6J × UL53–3) F1 hybrid mice were randomized into four groups, two groups of p53wt/wt mice and two groups of p53val135/wt mice. Control groups 1 and 2 were given 0.1 ml PBS i.p. once weekly for 10 weeks. Test groups 3 and 4 were given the carcinoxin DMH (20 mg/kg body weight) once weekly for 10 weeks (i.p. injection of DMH in 0.1 ml PBS). All of the animals were observed for clinical signs of illness. One half of all four groups were euthanized by CO2 asphyxiation at 7 months and the other half at 9.5 months after DMH exposure. A gross necropsy was performed; all of the organs, including colon, intestine, lung, liver, kidney, uterus, and testes, were macroscopically examined for tumors. In the case of uterine tissues and tumors, the normal uteri were aged and stage-matched (at late proestrus stage of the menstrual cycle) with the uterine tumors. The tissues were then fixed in 10% neutral-buffered formalin overnight, followed by 70% ethanol and paraffin-embedding. Tissue sections (5 μm) were stained with H&E for histopathological examination.

**Semi quantitative RT-PCR.** Total RNA was isolated from mouse uterine sarcomas or normal tissues using the TRIzol reagent (Life Technologies, Inc.). Total RNA (2 μg) was used to synthesize cDNA in a total reaction volume of 40 μl. After incubating the RNA in diethylpyrocarbonate-treated water at 70°C for 10 min, the following components were added: 1.5 μg oligo(dT), 8 μl of 5× reaction buffer [250 mM Tris-HCl (pH 8.3); 375 mM KCl, and 15 mM MgCl2], 10 mM DTT, 40 units RNasin, and 300 units Moloney murine leukemia virus reverse transcriptase (Life Technologies, Inc.). The reaction mixture was incubated at 37°C for 1 h followed by termination the reaction at 95°C for 10 min. A 2-μl aliquot, added into 25 μl of total reaction mixture, was used to perform a quantitative RT-PCR analysis. Table 1 lists the sequences of PCR primers for p53 downstream target genes. The PCR reaction mixtures included 0.2 mM deoxynucleotide triphosphates, 1.5 mM MgCl2, 1 μM downstream gene-specific primer, 1 μM upstream gene-specific primer with [γ-32P]ATP end-labeled, and 0.025 unit Taq DNA Polymerase (Promega, Madison, WI), and this mixture was subjected to 25 cycles of amplification at 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min. A pair of primers specifically flanking a fragment of GAPDH gene was also co-amplified as an internal control. The forward primer of GAPDH was end-labeled with [γ-32P]ATP by T4 polynucleotide kinase. The PCR products were resolved in an 8% denaturing polyacrylamide gel, and the results were quantitated by densitometry using a Shimatzu Dual-wavelength thin-layer chromatography scanner CS-930 and by densitometry using ImageQuant software.

**Northern Blot Analyses.** Total RNA was isolated from mouse uterine sarcomas or normal uterus using the TRIzol reagent (Life Technologies, Inc.). Northern blot analysis on uterine samples was performed to confirm the data from RT-PCR analysis. Total RNA (30 μg) was separated on a formaldehyde/ formamide-denaturing 1% agarose gel and subsequently transferred to a nitrocellulose membrane, and hybridized to 32P-labeled probes. Autoradiographs were quantitated using a Bio-Rad densitometer. The relative amount of RNA in each sample was normalized to that of GAPDH mRNA to correct for differences in sample loading and transfer. The cDNA probes were generated by PCR using the primer sequences in Table 1.

**Chemoprevention Study in Mice Treated with DMH.** Six-week-old female (C57BL/6J × UL53–3) F1 hybrid mice were randomized into four groups, two each of p53wt/wt mice and p53val135/wt mice. AIN-76A-purified diet was fed to mice in Groups 1 and 2 throughout the study. Groups 3 and 4 received the piroxicam diet (100 ppm) in AIN-76A diet beginning 1 week before administration of DMH and throughout the study. The diet containing piroxicam was made weekly, and fresh diet was provided to the animals three times per week. Starting at 7 weeks of age, all of the mice were treated with DMH (i.p., 20 mg/kg body weight in 0.1 ml PBS) once weekly for 10 weeks. One half of each study group was euthanized at 7 months and the remaining 9.5 months after DMH exposure. All of the mice were necropsied, and tissues were fixed in 10% neutral-buffered formalin overnight followed by 70% ethanol and paraffin embedding. Tissue sections (5 μm) were stained with H&E for histopathological examination.

**Statistical Analysis.** Student’s t test was used to determine the difference in incidence and multiplicity of tumors between control and treated groups.

**RESULTS**

**Effect of p53 Germ-Line Mutation (Ala 135 Val) on DMH-induced Tumorigenesis.** One of the most striking observations from this experiment was the dramatic increase in the induction of uterine sarcomas in p53val135/wt mice. Whereas the incidence of uterine sarcomas in DMH-treated p53wt/wt mice was ~10%, ~90% of the p53val135/wt mice had uterine sarcomas (Table 2). Morphologically, uterine lesions were grossly enlarged (up to 10 g in weight) and hyperemic occupying the pelvis and part of the abdominal cavity. Tumor size varied from 1 to 4 cm in diameter, and tumor weight from 5 to 10 g. In all of the cases, the whole uterus was diffuse thickening, and uterine horns were grossly dilated. In some cases, the uterine cavity was filled with slightly opaque fluid. Enlarged uterine sarcomas could sometimes be easily palpated through the abdominal wall and protruded through the vagina. As shown in Fig. 1, histologically most of the uterine sarcomas were leiomyosarcoma, which was composed of the tumor cells with large nuclei and associated with abnormal mitosis (>50 mitosis cell/50 high-power fields). The gross morphology and histological appearance of uterine sarcomas were similar in p53wt/wt and p53val135/wt mice. No uterine tumors were found in either the p53wt/wt or p53val135/wt control mice. Because spontaneous uterine tumors are generally seen in these mice at 24 months, we could not

**Table 1 PCR primers used for RT-PCR analyses and to generate cDNA probes for Northern blot analyses of p53 downstream target genes**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sequences (5’→3’)</th>
<th>Size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>p21</td>
<td>ATGCCAATCCTGTGATGT</td>
<td>302</td>
</tr>
<tr>
<td>Gadd45</td>
<td>TGCCAGGGCACAGAAGAGT</td>
<td>317</td>
</tr>
<tr>
<td>14-3-3</td>
<td>TAGTACCAAGAACAGGTTG</td>
<td>300</td>
</tr>
<tr>
<td>Reprimo</td>
<td>TGAGTTGGCTGCTTTGTGC</td>
<td>310</td>
</tr>
<tr>
<td>PC3/TIS21/BTG2</td>
<td>ATGCCCGTCACCATCTTGA</td>
<td>466</td>
</tr>
<tr>
<td>B99</td>
<td>CCTACGGTACAGAGAAGAG</td>
<td>384</td>
</tr>
<tr>
<td>PERP</td>
<td>AACGATCTCGACATCGTC</td>
<td>265</td>
</tr>
<tr>
<td>NOXA</td>
<td>CTTGAGTCGACCGTCAACTC</td>
<td>250</td>
</tr>
<tr>
<td>KILLER/DR5</td>
<td>CGGTATACTAATTGGAAGCC</td>
<td>281</td>
</tr>
<tr>
<td>FAS</td>
<td>CCAGGAATTTTAAAGCTGAGG</td>
<td>320</td>
</tr>
<tr>
<td>Bax</td>
<td>GACAAGCGCTGAGTAAGTC</td>
<td>186</td>
</tr>
<tr>
<td>PIDD</td>
<td>AGCATATATCGGAGTCCAG</td>
<td>284</td>
</tr>
<tr>
<td>BAI1</td>
<td>CAAAGACTCGAAGCTAGTC</td>
<td>291</td>
</tr>
<tr>
<td>TSP1</td>
<td>GAGACATCGTGGACAAGAC</td>
<td>256</td>
</tr>
<tr>
<td>Maspin</td>
<td>GACAGACTAATGCAAGAAG</td>
<td>295</td>
</tr>
</tbody>
</table>

The p53wt/wt mice were randomized into four groups, two groups of p53wt/wt mice and two groups of p53val135/wt mice. No uterine tumors were found in either the p53wt/wt or p53val135/wt control mice. Because spontaneous uterine tumors are generally seen in these mice at 24 months, we could not
determine whether the p53 transgenic mice have increased susceptibility to ‘spontaneously occurring’ uterine tumors.

Next, we examined the expression of 15 known p53 downstream target genes in the normal uteri and uterine sarcomas from p53 val135/wt and p53 wt/wt mice using semiquantitative RT-PCR and Northern blotting. Fifteen p53 target genes were examined to identify possible target(s) of p53 that mediated the tumor-enhancing effect. The genes examined included genes that inhibit cell-cycle progression (p21, Gadd45, 14–3–3–3/HT268, PC3/TIS21/BTG2, B99, and Reprimo; Ref. 13). Five uterine sarcomas and a normal uterus from p53 val135/wt mice, and six uteri from p53 wt/wt mice were examined. The expression of Reprimo mRNA was significantly higher in the normal uteri in p53 wt/wt mice when compared with the normal uterus and uterine sarcomas in p53 val135/wt mice (Fig. 2). The remaining 14 p53 target genes did not exhibit significant changes in mRNA expression in uterine tissues from p53 val135/wt versus those from p53 wt/wt mice (data not shown). These results suggest that the observed increase in mouse uterine sarcomas in p53 val135/wt transgenic mice may be mediated by the inability of the p53 val135wt dominant-negative mutant to induce expression of Reprimo, a cell cycle regulator. Furthermore, we have used only one pair of uteri from untreated mice (p53 val135/wt and p53 wt/wt) and the result is rather preliminary. Thus, additional experiments would be required to determine whether the deregulation of Reprimo is contributing to tumorigenesis.

We observed a significant increase in tumorigenesis in the colon, lung, and liver of p53 val135/wt mice. The incidence of colon tumors in DMH-treated p53 wt/wt mice (male and female) was 39%, with an average multiplicity of 0.39 tumors/mouse. DMH treatment of
p53<sup>val135</sup> wt/mice produced an average colon tumor incidence of 57% and multiplicity of 0.77 tumors/mouse. Colon tumors were confirmed as adenocarcinomas by light microscopic examination of H&E-stained sections. These data indicate that colon tumor multiplicity was significantly higher in p53<sup>val135</sup> wt mice (Table 2, group 4) versus p53<sup>wt</sup> wt mice (Table 2, group 3). Mice carrying the mutant p53 transgene (135Val) developed a higher number of lung tumors (0.56 tumors/mouse; Table 2, group 4) after treatment with DMH than wild-type mice (0.15 tumors/mouse; P < 0.0001; Table 2, group 3). In addition, the p53 transgene predisposes mice to liver tumor development, because nearly 10% of p53<sup>val135</sup> wt mice developed hepatomas after exposure to DMH, whereas only 1% of the p53<sup>wt</sup> wt mice developed hepatomas.

**Chemopreventive Effect of Piroxicam on DMN-induced Tumorogenesis in p53 Transgenic Mice.** As shown in Table 3, administration of piroxicam significantly reduced the incidence and multiplicity of DMN-induced colon tumors in both p53<sup>val135</sup> wt and p53<sup>wt</sup> wt mice without affecting tumor occurrence in the uterus, lung, or liver. The incidence of colon tumors in DMH-treated p53<sup>val135</sup> wt mice was 33%, with an average multiplicity of 0.33 tumors/mouse. In p53<sup>val135</sup> wt mice, the colon tumor incidence was 61% with an average multiplicity of 0.74 tumors/mouse. The incidence of colon tumors in the DMH + piroxicam-treated p53<sup>wt</sup> wt group was 5%, with tumor multiplicity of 0.06 tumors/mouse. The corresponding incidence in treated p53<sup>val135</sup> wt mice was 22%, with an average multiplicity of 0.26 tumors/mouse. These data indicate that piroxicam greatly inhibited colon tumor development in both p53<sup>wt</sup> wt and p53<sup>val135</sup> wt mice. In contrast, piroxicam failed to alter the incidence of lung tumors, liver tumors, or uterine sarcomas in p53<sup>val135</sup> wt mice or p53<sup>wt</sup> wt mice.

**DISCUSSION**

Somatic mutations in the p53 tumor suppressor gene are known to occur at high frequency in cancers from multiple organ sites including breast, lung, colon, bladder, and prostate (1). People with germ-line p53 mutations (LFS) develop various types of cancer including sarcomas, breast, lung, brain, and adenocortical tumors, and leukemia (4, 5, 34, 35). These results suggest that p53 plays a critical role in the predisposition to and progression of multiple cancer types. In the present study, we used a p53 transgenic mouse cross-bred with C57BL/6J mice to analyze the effects of a germ-line p53 mutation on tumor development in multiple organs. The results demonstrating increased numbers of lung tumors after treatment of mutant p53 mice with DMH agrees with our previous data (33). These data showed that (A/J × UL53–3) F1 mice containing a mutant p53 gene were more susceptible to lung adenoma induction by a variety of agents including the tobacco-related carcinogens 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol and benzo(a)pyrene (33). The present study also shows that the introduction of a p53 mutation (Ala135Val) into the germ-line significantly enhances tumorigenesis in the uterus, colon, and liver. The results are more striking in the uterus and lung than in the colon. The fact that uterine sarcomas and lung tumors are readily induced agrees with the known high frequency in individuals with LFS of both sarcomas and lung cancers. In contrast, a germ-line mutation in p53 did not affect chemically induced urinary bladder tumorigenesis. These data demonstrate that the effect of the p53 transgene is organspecific. Our finding that (C57BL/6J × UL53–3)F1 p53 mutant mice (val135) are highly susceptible to the chemical induction of various tumors should provide an important mouse model in which to study the role of p53 in tumorigenesis, to evaluate potential preventative or therapeutic agents, and finally as a model for testing potential genotoxic carcinogens.

As presented in the “Introduction,” we thought that mice with a dominant-negative mutation in the p53 tumor suppressor gene appeared to be a logical next step beyond the use of heterozygous knockout mice for mechanistic studies, because these animals more closely mimic the human situation. Although previous reports showed that mice heterozygous for knockout of the p53 gene were not more susceptible to chemically induced tumors of the lung, colon, and liver (36–39), they have been shown to be more susceptible to a variety of other chemically induced tumors including OH-BBN-induced urinary bladder carcinogenesis. Interestingly, we found that mice with a germ-line mutation in p53 had roughly the same incidence and multiplicity of urinary bladder tumors after treatment with OH-BBN as wild-type mice. The mechanism for the high sensitivity of the p53 mutant mice (val135) to tumor induction in many organs is not clear at present. The possible contributing factors include genetic backgrounds of the mice used, the position effects because of the integration site of the transgene, effects of organ-specific expression of the transgene, and the gain-of-function activities of the transgene. A recent report showed that expression of mutant p53 in p53-null cells enhanced malignant transformation, indicating that p53 mutations can promote tumorigenesis above the level seen in p53-null cells (40, 41). Increasing evidence supports the existence of gain-of-function activities in cells carrying certain missense p53 mutations (42–47). It is conceivable that the Val135 mutant can exert oncogenic functions in different target tissues independently of the inactivation of wild-type p53. Additional studies are under way to compare the role of p53 null mutations (heterozygous) and the p53 Val135 transgene on carcinogenesis in mice of the same strain.

Perhaps the most striking observation in this study is the profound effect of the p53 transgene on development of uterine sarcomas in female mice. This finding is in agreement with the finding in individuals with LFS of a high incidence and early onset of sarcomas. Our data show that p53<sup>val135</sup> wt mice, as compared with wild-type mice, exhibited a high incidence of uterine sarcomas in response to DMH, strongly indicating that uterine sarcoma formation is highly p53-dependent. When 15 p53 downstream target genes were examined, expression of a cell cycle regulator, Reprimo, was significantly decreased in uterine tissues of p53<sup>val135</sup> wt mice as compared with those from p53<sup>wt</sup> wt mice. Reprimo, a newly discovered p53 target gene, is...
induced by X-ray-irradiation and is involved in G2 arrest of the cell cycle (48). Expression of Reprimo mRNA can be induced by ectopic p53 expression. Reprimo is a highly glycosylated protein and is localized in the cytoplasm (48). Overexpression of Reprimo in HeLa cells resulted in G2 arrest by affecting the Cdc2/cyclin B1 regulation pathway (48). In fact, Reprimo regulates cell-cycle arrest in a p53 dependent manner (48, 49). Thus, we hypothesize that on exposure of p53val135/wt mice to DMH, the wild-type p53 is not induced or activated because of the dominant negative effect of the mutant transgene, which results in a lowered expression of Reprimo presumably leading to cell proliferation. In contrast, high levels of Reprimo are found in p53wt/wt mice because of the presence of wild-type p53 protein resulting in cell-cycle arrest. These results suggest that reduction of p53-dependent cell-cycle inhibition might contribute to the observed increase in the incidence of uterine sarcomas in p53val135/wt mice.

We also observed that (C57BL/6J × UL53–3) F1 p53 mutant mice carrying a p53 transgene (Ala135) exhibited an increased susceptibility (3.7-fold) to DMH-induced lung cancer in a lung tumor-resistant strain of mice. This result is consistent with our recent finding that the same germ-line p53 mutation caused a 3–4-fold increased susceptibility to chemically induced lung tumorigenesis in a lung tumor-susceptible mouse background [(A/J × UL53–3)F1 mice (33)]. Taken together, these results indicate that the p53 germ-line mutation exhibits an enhanced susceptibility to lung tumorigenesis independent of genetic background of the mice (50). Lung cancer is highly prevalent in males from families with LFS, and somatic p53 mutations have been found in 50–80% of sporadic lung cancers (1–12, 33). These results suggest that p53 plays a critical role in the predisposition and development of human lung cancer. This would appear to make the lung model particularly relevant for investigating the effects of preventative or therapeutic agents. In addition, it may make these mice particularly useful in screening for potential lung carcinogens and examination of the effects of cigarette smoke. In fact, our initial studies showed that two preventative agents dexamethasone and green tea were equally effective in preventing adenoma formation in (A/J × UL53–3) F1 mice with or without a mutation in p53 (33).

Although p53 mutations are relatively common in human colon cancer and although they appear to arise during the later stages of tumor progression, the increased levels of colon cancer were not profound. Interestingly, colon cancer is not extremely high in individuals with LFS. We investigated the efficacy of piroxicam as an inhibitor of DMH-induced carcinogenesis. On the basis of its efficacy in a variety of rodent colon model systems, the NSAID piroxicam was used. When the study was initiated we expected to see primarily colon cancer after treatment with DMH. In fact, the preponderance of colon tumors was observed in p53val/wt mice treated with DMH (Table 2, Group 3). Piroxicam significantly inhibited the formation of colon tumors in both p53val/wt mice and p53val135/wt mice. However, it failed to prevent the induction of uterine sarcomas, lung adenomas, or hepatomas. These observations suggest the following points. First, piroxicam is highly effective against colon tumors with or without a p53 mutation. The efficacy of piroxicam in colon tumors with a p53 mutation parallels previous studies demonstrating the efficacy of indomethacin and a COX-2-specific inhibitor, Celecoxib, in an UV-induced mouse skin tumor model (51). In that model, virtually all of the skin tumors have mutations in the p53 gene (51). These studies support previous findings in mice and rats (28, 52), demonstrating that piroxicam is an effective chemopreventive agent in rodents. Our results support the use of NSAIDs for individuals at high risk for colon cancer, irrespective of p53 status. Second, the effects of NSAIDs are tissue-specific. Despite being relatively effective in inhibiting colon tumors, piroxicam was ineffective in inhibiting uterine, liver, or lung tumors in mice with a mutant p53.

The present studies demonstrate that in situ tumors with a germ-line p53 mutation can be induced in mice in a variety of organ sites, including lung, liver, colon, and uterus. More recently, we have found that this mouse model is highly susceptible to chemically induced skin tumors and that the mutant p53 transgene synergistically interacts with an activated H-ras transgene.6 This model, using the dominant-negative p53 mutation, is particularly appealing because it is effective in the heterozygous state. This will allow its use in F1 mice from susceptible inbred strains of mice and allow one to mate to transgenic mice with or without a mutation in the p53 gene. One possible limitation of this model is that the mutant transgene is located in an ectopic chromosomal location with possible effect on tumorigenesis, and “knock-in” p53 transgenic mice would probably be more ideal.

ACKNOWLEDGMENTS

We thank Gary Stoner and Elizabeth Wiley for critical reading of this manuscript. Some of the work was performed at Medical College of Ohio (Toledo, OH). We thank Clinton J. Grubbs and Amy S. Anderson for sharing their unpublished information.

REFERENCES


6 Z. Zhang and M. You, unpublished data.


p53 Transgenic Mice Are Highly Susceptible to 1, 2-Dimethylhydrazine-induced Uterine Sarcomas

Zhongqiu Zhang, Jie Li, Laura E. Lantry, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/62/11/3024

Cited articles
This article cites 49 articles, 26 of which you can access for free at:
http://cancerres.aacrjournals.org/content/62/11/3024.full.html#ref-list-1

Citing articles
This article has been cited by 5 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/62/11/3024.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.