Tissue-specific Induction of p53 Targets in Vivo

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ABSTRACT

The in vivo response to radiotherapy is not well understood but appears to involve the p53 tumor suppressor protein. We investigated the expression of apoptosis-inducing p53 target genes during γ-irradiation-induced cell death in p53+/− or p53−/− mice using in situ hybridization. Our results reveal striking tissue specificity with distinct regulation of target p53-induced genes in different cells and tissue compartments, as well as variations in dependence on p53 for basal expression. p53-dependent induction of Puma occurred in the splenic white pulp, whereas Noxa and Bid were induced in the red pulp. These patterns correlated with activation of caspase-3 in both compartments. All apoptotic targets of p53 studied here (DR5, Bid, Puma, Noxa) were induced in the jejunum and ileum, which appeared to be the tissues most sensitive to irradiation. We also observed unexpected differences in p53 target gene activation between the transverse and descending colon. Finally, in the liver where irradiation did not lead to caspase-3 activation, we primarily observed p21WAF1 induction as the major p53-dependent target gene response. Our findings indicate that the selectivity of p53 in transactivation following DNA damage in vivo results in unique tissue and cell type specificity, which may correlate with growth arrest or variable sensitivity to γ-irradiation.

INTRODUCTION

wt533 protein exerts inhibitory effects toward the growth of abnormal cells and has been considered a guardian of the genome in preventing cancer development (1–4). This concept is supported by the fact that the p53 gene is the most common target for mutation in human cancer (5), that p53 knockout mice show a high incidence of tumor development (6), and that germ-line mutation of one p53 allele in humans gives rise to the Li-Fraumeni cancer-susceptibility syndrome (6). wt53 is a short-lived protein with a rapid turnover under normal unstressed conditions. The precise mechanism by which p53 is activated by cellular stress is not completely understood. Upon genotoxic insult, a rapid stabilization of the p53 protein and its activation leads to cell cycle arrest and/or apoptosis; the arrest allows cells to repair damaged DNA, whereas apoptosis removes damaged cells from the replicative pool to maintain genome integrity (1, 7). The biochemical function of p53 that best explains its effects is its sequence-specific transcriptional activity that transactivates target genes through binding a consensus motif in their genomic DNA sequences (7–11).

The ability of p53 to promote cell cycle arrest is well understood in terms of its ability to transactivate three critical target genes: p21WAF1, GADD45, and 14-3-3σ (12–14). p21WAF1 protein binds to and inactivates cyclin-dependent kinases, arrests cells in G1 and prevents S-phase entry. GADD45 and 14-3-3σ appear to be involved in control of the G2/M transition (15, 16). A number of p53 target genes with proapoptotic activity have been identified. They fall into three groups based on their subcellular location (17). The first group of genes encode proteins that localize to the cell membrane (e.g., CD95, KILLER/DR5, PERP). The KILLER/DR5 and CD95 (Fas/APO-1) proteins are two unique members of the tumor necrosis factor receptor superfamily that are induced by DNA damage in a p53-dependent manner and appear to be sufficient to induce apoptosis in some systems (18–21). PERP is a plasma membrane protein whose induction by doxorubicin is correlated with activation of the p53-dependent apoptotic pathway in transformed mouse embryonic fibroblasts (22). The second group of genes encode proteins that localize to the cytoplasm, including PIDD and PIGs. PIDD can be up-regulated by γ-irradiation through a transcriptional mechanism (23). PIGs (p53-induced genes) have been found to be involved in apoptosis by generating or responding to oxidative stress (24). The third group of genes encode proteins that localize to the mitochondria (e.g., Bak, Noxa, Puma, p53Aip1). Bak, the best characterized mediator of p53-dependent apoptosis, translocates to the mitochondria in response to DNA damage and, in turn, induces cytochrome c release from the mitochondria (25). Both Noxa and p53Aip1 are dependent on p53 for induction following DNA damage. Furthermore, p53Aip1 induction in response to DNA damage correlates with the phosphorylation of p53 at serine 46 and apoptosis induction (26, 27). Puma expression inhibits cell growth and rapidly induces apoptosis through a pathway involving cytochrome c release and activation of caspases 9 and 3 (28, 29). Bid was very recently found to be a p53 target and may contribute to chemosensitivity (30).

p53+/− and p53−/− mice have been used to study the roles of p53 itself and its previously defined targets in radiosensitivity in vivo (31–37). p53 null-mice have been found to be resistant to apoptosis induced by γ-irradiation in the developing nervous system (35), spleen, thymus (36), and the small intestine (31, 36, 37). Additionally, p53-null mice have been found to be resistant to the apoptosis triggered by 5-fluorouracil in small intestine (38, 39) by 1-β-β-arabinofuranosylcytosine in sympathetic neurons (40) and by adriamycin in the thymus, spleen, and small intestine (36). The activity of the p53 apoptotic pathway varies widely between tissues. A systematic investigation p53 target gene induction in vivo is of interest because it may lead to strategies for possible interference with expression. In addition, understanding the patterns of gene induction in vivo may help with the elucidation of pathways of cross-talk between factors affecting cell fate after irradiation.

To date, although a large number of p53 targets have been identified as candidate effectors of p53-dependent apoptosis, none of them appears to be a principal mediator of the p53 apoptotic signal. We hypothesized there may be coordinate regulation of apoptotic targets that may ultimately correlate with radiosensitivity. In the present studies, one representative target of p53 function in growth arrest, p21WAF1 (12) and four recently identified p53 target genes functioning in cell death/apoptosis (KILLER/DR5, Bid, Noxa, and Puma; Refs. 20, 26, 28–30) were systematically studied. We found that γ-irradiation-induced p53 transactivation leads to some apparent
overlapping as well as unique tissue/cell type specificity. The observed patterns may correlate with caspase-3 activity in the corresponding tissues and provide some insights into the variable sensitivity of tissues to γ-irradiation.

MATERIALS AND METHODS

Animals and γ-Irradiation. Five- to six-week-old female p53+/– and p53−/− mice were obtained from Jackson Laboratories. Two p53−/− mice and two p53−/− mice received total body γ-irradiation using a dose of 5 Gy, whereas an additional two p53−/− mice and p53+/− mice were used as experimental controls with no treatment. The mice were euthanized 6 h later using an approved Institutional Animal Care and Use Committee Protocol, which followed recommendations of the Panel on Euthanasia of the American Veterinary Medical Association. Thymus, spleen, liver, duodenum, jejunum, ileum, transverse colon, and descending colon were harvested, fixed in 4% paraformaldehyde overnight at 4°C and paraffin embedded.

Probe Preparation and In Situ Hybridization. IMAGE clones of mouse Puma and Noxa purchased from ResGen Invitrogen Corporation were prepared to generate mouse Puma and Noxa cDNA fragments. Approximately 500-bp PCR products of mouse p21WAF1 (12) mouse KILLER/DR5 (21), mouse Bcl (30), mouse Puma, and Noxa were cloned into the Topo-TA vector (Invitrogen) with both sp6 and T7 promoters in order to make either antisense or sense RNA probes with a Dig RNA labeling kit (SP6/T7; Roche). The subcloned cDNA fragments were sequenced to confirm the authenticity of the inserts before their use to generate in situ RNA probes. In situ hybridization was performed as described previously (41).

Immunohistochemistry. Routine 5-μm paraffin sections were prepared. Deparaffinized sections were heated for 15 min in 0.01 M citrate buffer (pH 6.2) in a microwave oven for antigen retrieval. The sections were then immunostained using the ABC peroxidase method followed by a weak hematoxylin counterstain. In some cases, 0.5% methyl green was used as the counterstain. The primary antibodies used were those that could detect active mouse Caspase-3 (Novacastra), mouse Ki67 (Novacastra), or mouse p53 (Santa Cruz Biotechnology). The TUNEL assay was performed using the ApopTag Plus Peroxidase in Situ Apoptosis Detection Kit (Intergen, Purchase, NY) following the protocols provided by the manufacturer.

RESULTS

Variable Levels of p53-dependent Apoptotic Activity in γ-Irradiated Tissues. The p53 tumor suppressor pathway is a pivotal mediator of genotoxic stress responses. These responses ultimately protect the organism from accumulating genetically altered and potentially cancerous cells by inducing growth arrest and/or apoptosis in damaged cells (3, 4). To better define the relationship between the apoptotic response to γ-irradiation and p53 status/p53 target gene activation, we systematically measured caspase-3 activity in various tissues of control and irradiated (5 Gy) wild-type or p53-null mice. As expected from previous studies that have measured DNA fragmentation, poly(ADP-ribose)polymerase cleavage, or TUNEL staining in tissues, the induction of cell death after γ-irradiation as measured by the active caspase-3 assay appears to require the presence of wtp53. wtp53-containing thymus, spleen, duodenum, jejunum, ileum, transverse colon, and descending colon all induced caspase-3 activity to differing extents after irradiation (Fig. 1). The observed cell death induction was correlated with increased p53 protein expression after radiation, although there was no obvious correlation between the magnitude of p53 stabilization and the degree to which caspase-3 was activated (Figs. 1 and 2). p53−/+ jejunum- and ileum-activated caspase-3 in response to γ-irradiation to extremely high levels as compared with other tissues. In contrast, the level of caspase-3 activity was too weak to be detected in liver tissue sections. To confirm these two extremes of possible response (ileum/jejunum versus liver), we performed TUNEL assay and analysis of the proliferation marker ki67 in irradiated jejunum, ileum, and liver. We detected a strong signal by TUNEL assay in irradiated p53−/+ jejunum and ileum (Fig. 3A) coupled with decreased ki67 expression (Fig. 3B). In the liver, we detected a weak signal after irradiation by TUNEL assay and a strong signal for ki67 expression. Thus, the two extremes in caspase-3 activity between liver and small bowel have been confirmed by an independent measure of cell death in vivo. In summary, p53 is required for irradiation-induced apoptosis in vivo, and the level of p53-dependent apoptosis appears to vary widely in a tissue-specific manner with a minimum apoptosis observed in the liver, and a maximum death observed in the small bowel.

p53-dependent p21WAF1 Induction by γ-Irradiation in Liver and Other Tissues Inversely Correlates with the Degree of p53-dependent Apoptosis in Vivo. The role of p53 protein in both cell proliferation and apoptosis is mainly mediated through its ability to transactivate target genes. p21WAF1 (12, 42) is a well-known mediator of p53 function in cell cycle arrest in response to DNA damage. Moreover, numerous studies have observed that p21WAF1 exerts a protective effect toward cell death and that deletion of p21WAF1 confers sensitivity to a number of apoptotic stimuli. To systematically gain insight into the radiation response in vivo and possible influence of p21WAF1 on death versus arrest responses, we examined p21WAF1 expression by in situ hybridization in tissues from irradiated or control wild-type and p53-null mice. Fig. 4 and Table 1 show that p21WAF1 was strongly induced by γ-irradiation in p53−/+ liver (Fig. 4A) and descending colon (Fig. 4C) and slightly in p53−/+ thymus (Fig. 4B), spleen (Fig. 4B), duodenum (Fig. 4C), ileum, and transverse colon (Fig. 4A), but there is no induction by γ-irradiation in p53−/- tissues studied here, except for a slight induction in p53−/- thymus and spleen (Fig. 4B) and no induction in p53−/+ jejunum (Fig. 4C). p21WAF1 basal expression was notably high in p53−/- ileum, jejunum, and descending colon (Fig. 4, A and C). This is consistent with previous observations regarding p53-independent p21WAF1 expression in colon epithelial (42). Interestingly, the level of p21WAF1 expression was much higher in nonirradiated p53−/- descending colon as compared with p53−/+ descending colon (Fig. 4C). As a control, no signal was detected from tissue sections after hybridization with the sense p21WAF1 probe (data not shown). Taken together with the data shown in Fig. 1 and Table 1, the strongest p21WAF1 induction after γ-irradiation occurred in tissues with the least or absent p53-dependent apoptotic activity. Thus, p21WAF1 induction in tissues in response to γ-irradiation is generally p53-dependent and varies in a tissue-specific manner, which appears to inversely correlate with the level of p53-dependent apoptotic activity occurring in the corresponding tissues.

wtp53-dependent KILLER/DR5 Induction in Response to γ-Irradiation in Thymus, Spleen, and Transverse Colon. KILLER/DR5 was identified as a proapoptotic member of the tumor necrosis factor-related apoptosis-inducing ligand receptor family in a screen to find p53 targets up-regulated in chemosensitive but not chemoresistant ovarian carcinoma cells (20). We therefore investigated KILLER/DR5 expression by in situ hybridization using unirradiated and irradiated tissues from the wild-type and p53-null mice. We found (Fig. 5 and Table 1) that in p53−/− and p53−/+ descending colon, p53−/− and p53−/+ ileum, and p53−/+ thymus, spleen, transverse colon, and descending colon, KILLER/DR5 is slightly induced by γ-irradiation but not in other p53−/− or p53−/+ tissues examined (liver, duodenum, jejunum). KILLER/DR5 basal expression appears in all tissues but the thymus (Fig. 5A), with a wtp53-dependent induction in duodenum (Fig. 5B). In the thymus (Fig. 5A), there was a greater induction of DR5 in p53−/+ tissue as compared with p53−/−. In contrast, KILLER/DR5 expression was increased by irradiation and did not correlate with wtp53 status in the ileum (Fig. 5B) and descending colon (Fig. 5A). These results indicate that KILLER/DR5 can be expressed in a
p53-dependent or -independent manner and that the p53 dependence varies in a tissue-specific manner (in situ hybridization by using sense DR5 probe was also performed, and no signal was detectable; data not shown). The mechanism of p53-independent KILLER/DR5 induction in vivo in the thymus (Fig. 5A), duodenum (Fig. 5B), and descending colon (Fig. 5A) remains unclear. Possibilities include a potential role for p53 family members such as p63 or p73 or pathways that are independent of the p53 family. It should be noted, however, that the major induction of KILLER/DR5 after γ-irradiation in the thymus and spleen (Fig. 5A) is p53-dependent, and in the transverse colon (Fig. 5A), KILLER/DR5 induction by irradiation appears to occur exclusively in a p53-dependent manner.

**Bid Expression Depends on wtp53 after γ-Irradiation in Spleen, Thymus, and Transverse Colon.** Bid is a BH3 homology domain-containing protein that acts as a bridge in apoptotic signaling between the extrinsic death receptor pathway and the mitochondrial pathway (43). Bid was recently identified as a p53 target gene (30) and was suggested to contribute to chemosensitivity. Thus, Bid was chosen as a target to further identify correlates between p53 target gene induction and irradiation-induced apoptosis in vivo. Fig. 6 and Table 1 show that among all p53-null tissues, Bid expression is detectable in irradiated jejunum and thymus. Among all p53+/- tissues, Bid induction by γ-irradiation shows a readily detectable increase in the transverse colon (Fig. 6A) and a more moderate increase in the thymus (Fig. 6B) and spleen (Fig. 6A). Bid basal expression, interestingly, is very strong in p53+/- jejunum with no detectable difference between nonirradiated and irradiated jejunum (Fig. 6C), indicating that Bid basal expression may depend on wtp53 but that induction by γ-irradiation may occur in the absence of p53 in this tissue. The signal from the sense probe was too weak to detect (data not shown). Thus, the dependence
Bid expression on wtp53 and its induction after irradiation appears tissue specific.

**Noxa Expression Is Strongly Induced in Thymus after γ-Irradiation.** Noxa (26) appears to be a candidate mediator of p53-dependent apoptosis as its induction after γ-irradiation in mouse cells depends on wtp53. To study Noxa regulation by p53 in vivo, we performed in situ hybridization to detect Noxa mRNA expression in tissues obtained from p53+/+ or p53−/− mice with or without γ-irradiation by using the antisense and sense Noxa probes simultaneously. We found that Noxa was induced by γ-irradiation in p53-null (or p53−/+ ) thymus, duodenum, jejunum, and transverse colon and in p53+/+ (but not p53-null) spleen (Fig. 7 and Table 1). The induction of Noxa following γ-irradiation is very strong in p53−/− thymus (Fig. 7B), suggesting a possible role for Noxa induction in the cell death that occurs in the thymus. Noxa expression was found to be relatively high in unirradiated duodenum (Fig. 7B), jejunum (Fig. 7B), and ileum (Fig. 7A) from either p53+/+ or p53−/− animals, as compared with the remainder of the untreated tissues that were examined. Data is not shown for the nondetectable Noxa sense signal. Therefore, Noxa, as a proapoptotic target of p53, is predicted to function mainly in the thymus, followed by spleen, presumably to mediate p53-dependent apoptosis after exposure to cellular stresses or DNA-damaging exposures such as γ-radiation. The basal expression of Noxa is relatively high in the small intestine and shows no obvious correlation with p53 status, suggesting possible p53-independent function in the small bowel.

**Dependence of Puma Expression on wtp53 in Spleen and Descending Colon and Localized Induction in White Pulp but Not Red Pulp of Spleen after γ-Irradiation.** Puma encodes a p53-up-regulated modulator of apoptosis, a member of the bcl-2 family, which may play a role in mediating p53-induced cell death through activation of mitochondrial cytochrome c release (28, 29). We investigated the basal expression of Puma as well as its induction after irradiation of wild-type and p53-null mice. We found no detectable induction of Puma expression by irradiation in any p53−/− tissue examined, except for duodenum and thymus (Fig. 8A). Puma induction was found to be dependent on the presence of wild-type p53 in spleen and descending colon and localized in white pulp but not red pulp of spleen (Fig. 8B). In the descending colon (DC), Puma was observed in the submucosal nerve plexus and in the muscularis propria, which is consistent with a role for Puma in mediating radiation-induced injury in the gut.
Fig. 3. Apoptotic death and growth arrest patterns in irradiated small bowel versus liver of wild-type and p53-null mice. A, TUNEL assay in mouse jejunum, ileum, and liver. ApopTag plus peroxidase kit was used. The apoptotic nuclei are stained brown. The brown nuclei appear only in p53+/−, irradiated jejunum and ileum and not in liver. B, immunostaining for Ki67 expression in mouse jejunum, ileum, and liver. The expressed Ki67 protein stains nuclei brown. Brown nuclei are extensively noted in unirradiated tissues, as well as irradiated p53+/− liver but not in irradiated p53+/− jejenum and ileum. Images of non-irradiated p53+/− tissues are similar to irradiated p53+/− tissues. Jej, jejunum.

Table 1. Patterns of p53 target gene expression in mouse tissues

<table>
<thead>
<tr>
<th>Gene</th>
<th>Thymus</th>
<th>Spleen</th>
<th>Liver</th>
<th>Duodenum</th>
<th>Jejunum</th>
<th>Ileum</th>
<th>Transverse colon</th>
<th>Descending colon</th>
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<tbody>
<tr>
<td>p21waf1</td>
<td>+ a</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
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<td>DR5</td>
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<td>+</td>
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<td>++++</td>
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<tr>
<td>Bid</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>ND</td>
<td>ND</td>
<td>+</td>
</tr>
<tr>
<td>Noxa</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+++</td>
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<tr>
<td>Puma</td>
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<td>+ b</td>
<td>−</td>
<td>−</td>
<td>+</td>
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</table>

a, −, not detectable; +, weak expression; ++, moderate expression; ++++, strong expression; ++++, stronger expression.

b Tissue compartment restriction (see Figs. 6, 7, and 8).

ND, not determined.

and Table 1). In contrast, in p53+/+ spleen (Fig. 8B), Puma was highly induced by irradiation in the white pulp, as compared with Noxa and Bid, the induction of which occurred in the red pulp of the spleen (Figs. 6A and 7A). Puma was also highly induced in p53+/+ thymus (Fig. 8C) and moderately induced in p53+/+ descending colon (Fig. 8B) by γ-irradiation. In the p53+/− jejunum (Fig. 8D), the basal level of Puma expression was higher in p53+/+ as compared with p53-null jejunum, and there was no induction observed after irradiation. Additionally, Puma was found to be expressed throughout the ileum (Fig. 8A) at similar levels between wild-type and...
p53-null mice. There was no induction of Puma in the ileum after irradiation (Fig. 8A), and no detectable sense probe signal in all tissue sections presented here (data not shown). These results indicate that the basal expression of Puma, induction of its expression in response to γ-irradiation, and dependence of its expression on wtp53 vary in a tissue-specific manner. p53-dependent induction of Puma in the splenic white pulp provides a clear example where even within a given tissue, a p53-induced target can be restricted to a compartment.

**DISCUSSION**

A number of recently discovered p53 target genes proposed as candidate mediators of DNA damage-induced apoptosis has been examined here for their induction during cell death in vivo. Our results provide critical new insights into the complexity of the radiation response within tissues in vivo. We recognized several patterns of gene expression by in situ hybridization using probes for p53 target genes, including the cyclin-dependent kinase inhibitor p21WAF1, as well as the proapoptotic genes KILLER/DR5, Noxa, Bid, and Puma.

The observed patterns include the following (see Table 1): (a) no detectable basal expression in either wtp53-expressing or p53-null tissues but strong induction after exposure to 5 Gy γ-radiation; (b) detectable basal expression in p53+/+ and p53−/− tissue and additional induction after irradiation; (c) detectable basal expression in p53+/+ and p53−/− tissue but without additional induction of gene expression after irradiation; (d) no detectable expression in either p53+/+ or p53−/− tissue and no induction after irradiation; and (e) induction of gene expression after irradiation in a p53-dependent manner that is restricted to a particular tissue compartment. In addition to the patterns of gene expression, we found evidence that the coordinated induction of certain p53 targets (or the lack thereof) may correlate well with whether apoptosis was observed and also with the degree to which a particular tissue underwent rapid or massive cell death in vivo. Finally, our study provides detailed comparisons showing remarkable differences in the patterns of gene expression before and after radiation at progressively distal locations within the gastrointestinal tract or within compartments of a given tissue such as the spleen.
In situ hybridization was used to reveal the cellular localization and relative level of expression of specific p53 target genes in tissue sections. The information thus derived about temporal and spatial expression and induction of the genes after irradiation suggests distinct in vivo situations where certain p53 targets may mediate apoptosis. Although Northern analysis and quantitative reverse transcription-PCR can identify the presence of a specific mRNA, as well as its level of induction, they do not provide information about the localization of the signal to specific cell populations in relation to tissue morphology (41). One of the major findings here is the specific compartmentalization of expression of *Bid*, *Noxa*, and *Puma* in irradiated spleen (Table 1, Figs. 6, 7, and 8) observed by in situ hybridization. In our study, in situ hybridization was performed among many kinds of tissues with five different probes. We optimized the hybridization conditions to maximize the signal with recognizable tissue structure before each probe was actually hybridized systematically. The level of gene expression in different tissues remains comparable because the tissue-specific conditions performed here were generated from the same standard (maximal signal with recognizable tissue architecture) in each pre-in situ hybridization.

This is, to our knowledge, the first detailed systematic examination of expression of a group of p53 target genes, including those recently discovered by in situ methodology in the in vivo response to γ-irradiation. One of the remarkable findings of this study is the apparent tissue specificity with which p53 selects targets for activation. In response to γ-irradiation in wtp53-containing tissue, *Noxa* was mainly induced in the thymus, *Puma* in white pulp of the spleen, *p21 WAF1* in the liver, and *Bid* in the transverse colon. Different parts of small and large intestines also showed different patterns of target gene expression and activation. Interestingly, only KILLER/DR5 and *Bid* were induced in the transverse colon, and a low level of *Puma* induction in the wtp53-containing descending colon was observed in response to γ-irradiation. We note that the basal expression of several genes appears to depend on wtp53, and those genes are not significantly induced by γ-irradiation. Examination of the tissue death response after irradiation revealed increased caspase-3 activity in all tissues but...
liver from irradiated p53+/+ mice as compared with the corresponding tissues from irradiated p53−/− mice or nonirradiated control p53+/+ or p53−/− mice. In the case of liver, we believe the apparently exclusive preference for p21 WAF1 activation, and not other p53 targets that could induce apoptosis, may, in part, explain the absence of detectable apoptotic death after radiation exposure. The p53 response profile and apparent lack of apoptosis in the liver does not exclude other types of toxicity such as necrotic death or inflammatory responses. The in situ hybridization results reveal remarkable variation in the selectivity of p53 for in vivo transactivation and the resulting gene expression patterns may correlate with the response to genotoxic stress.

γ-Irradiation induces a large variety of DNA lesions, including single- and double-strand breaks, base, and sugar damage (44, 45). The apoptotic pathway activated by γ-irradiation in proliferating cells is known to involve transcription of a variety of genes, among which the tumor suppressor gene p53 is one of the most relevant (46–48). Upon activation, p53 transactivates its target genes to induce cell growth arrest and/or apoptosis, responses that must be finely balanced in order to protect cells with intact genomes and at the same eliminate excess or damaged cells. The requirement of subtle regulation is reflected here by the complex tissue- and cell-specific responses. Our studies further expand previous work (31, 33, 34, 49, 50) examining p53-dependent apoptosis in tissues of spleen, thymus, and gut. We previously showed (34) that KILLER/DR5 and p21 WAF1 induction by γ-irradiation was p53 dependent in spleen, thymus, and small intestine and that there was some variation in the magnitude of gene induction using real-time reverse transcription-PCR-based measurements on bulk tissue mRNA. We have not only confirmed these observations but also provided extensive in situ studies on three new targets of p53 (Noxa, Puma, and Bid) along with KILLER/DR5 and p21 WAF1 in spleen, thymus, and liver, and we further investigated various portions of small and large intestines. Interestingly, the response to γ-irradiation in the spleen revealed evidence for p53-dependent cell death throughout the organ but various proapoptotic (BH3-domain containing) p53 targets were up-regulated in separate compartments; Bid and Noxa were expressed in the red pulp of the spleen, whereas Puma was induced in the white pulp. The molecular basis for compartmentalized regulation of specific p53 targets within a given tissue is not understood at present. In itself the compartmentalization suggests candidate
mediators of death in certain locations (because they are induced there) and also suggests that there may be unique functions of certain p53 targets in certain situations in vivo. Possible mechanisms for additional investigation for the selectivity of p53 for different targets in different tissue compartments within the spleen include (a) potential differences in p53 modification in the radiation response in different tissue compartments, and (b) potential tissue compartment-specific proteins that may positively or negatively regulate the selectivity of p53 for either particular DNA binding sequences or the transactivation of particular target genes.

In response to apoptotic signals, p53 protein is stabilized and activated, leading to transcriptional activation of multiple target genes that cause apoptosis of cells. These include death receptors, including Fas/Apo or KILLER/DR5 or proteins that are involved in mitochondria-mediated apoptosis, including Bax, Noxa, Puma, and p53Aip1. Activation of mitochondria-mediated apoptosis represents a major antitumor response of p53 (51). Among the identified target genes of p53, Bax encodes a proapoptotic Bcl-2 family member that can activate mitochondria-mediated apoptosis (25). However, in Bax-deficient mice, DNA damage-induced apoptosis occurs normally in thymocytes (52). Our data that Noxa was strongly induced by γ-irradiation in p53+/+ thymus may, in part, explain why apoptosis still occurs in Bax-deficient thymus.

The small intestine represents one of the most rapidly proliferating tissues of the body, with cell division occurring approximately every 5 min in each crypt (53). Despite its high proliferation rate, cancers rarely develop in the small intestine, suggesting that this tissue contains an efficient mechanism for regulating cell growth (54). Our data shows that all the proapoptotic p53 target genes studied here were to, at some extent, expressed in jejunum and ileum, and we suspect they may act synergistically to contribute to the strong wt/p53-dependent caspase-3 activation in jejunum and ileum after γ-irradiation. In contrast to the jejunum and ileum, there are less proapoptotic p53 targets expressed in the transverse and descending colon, which may correlate with the relatively low caspase-3 activity detected after irradiation. Liver is the only tissue studied here that did not display

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**Fig. 7. Tissue specificity and p53 dependence of Noxa gene induction after γ-irradiation in vivo.**

A. Noxa mRNA expression in mouse spleen, liver, descending colon, and ileum. Noxa mRNA expression is increased in irradiated p53+/+ red pulp of spleen but not in the irradiated p53−/− spleen. Noxa mRNA is expressed in the descending colon at higher levels in the ileum and is not additionally increased by irradiation in either of the p53+/+ or p53−/− corresponding tissues. The images of non-irradiated and irradiated p53+/+ tissues (data not shown) are similar to the nonirradiated p53−/− tissues. DC = descending colon. B. Noxa mRNA expression in mouse thymus, duodenum, jejunum, and transverse colon. Noxa mRNA expression is expressed in unirradiated thymus, increased additionally in irradiated p53+/+ thymus, and slightly increased in irradiated p53−/− thymus. Noxa mRNA expression is detectable in unirradiated duodenum and jejunum and is up-regulated to a similar extent in p53+/+ and p53−/− duodenum and jejunum, although the level of Noxa mRNA expression appears higher in irradiated jejunum versus duodenum. Noxa expression was not detectable in unirradiated transverse colon and was induced by irradiation regardless of p53 status. The images of nonirradiated p53+/+ tissues (data not shown) are similar to the nonirradiated p53−/− tissues. Duo = duodenum, Jej = jejunum, TC = transverse colon.
caspase-3 activation in the presence of wtp53 after γ-irradiation. This is in agreement with previous studies showing that liver does not undergo apoptosis after γ-irradiation (33, 55). However, significant p21WAF1 expression was induced in p53+/+ liver (Fig. 4), whereas none of the other proapoptotic targets studied here was significantly up-regulated. p21WAF1 seems to play an important role in determining whether a cell should undergo apoptosis or survive with p21WAF1 expression appearing to favor growth or growth arrest over cell death (56–59). Therefore, the pattern of p53 target gene expression in liver may explain, at least in part, the inability of γ-irradiation to induce caspase-3 activation, and this correlates with the resistance of liver to radiation-induced apoptosis.

The role of p53 in radiosensitivity is complex. In some cases, expression of wtp53 is associated with an increase in the sensitivity to anticancer treatment (60–63). Although loss of p53 function can also result in increased sensitivity to anticancer treatment in other situations (55, 64). In the current study, all patterns of gene expression demonstrate distinct tissue/cell type specificity as well as some overlap in gene expression induction of p53 targets, which seems to correlate well with the level of caspase-3 activation in particular tissues. Therefore, our study supports the concept that wtp53 contributes to radiosensitivity. It appears that induction of more proapoptotic target genes resulted in a stronger apoptotic response, e.g., in jejunum and ileum. In the future, additional p53 targets need to be analyzed, and in fact, it would be extremely useful to perform microarray analyses investigating global gene expression patterns in the radiation response of different tissues. In addition to the striking patterns of tissue specificity, the present studies provide a framework within which future studies can analyze p53 or proapoptotic targets of interest. The patterns of gene expression may suggest markers of tissue responsiveness to therapeutic manipulations and may provide essential clues to modulate tissue toxicity. In this regard, it will be of interest to determine whether tumors that arise from a given tissue maintain the genetically programmed p53 activation response profile as compared with the normal tissue of origin. If they do, one could envision scenarios where (transient) blockade of a proapoptotic p53 target not involved in the therapeutic response of a metastatic tumor may protect normal tissues exposed to chemotherapy or radiotherapy where the proapoptotic target gene induction contributes to toxicity. Another important direction that emerges from our studies involves additional work to understand the relationship, if any, between the observed strong DNA damage p53-mediated apoptotic response in the
small bowel of mice and the observed low incidence of intestinal tumors in humans. We hypothesize that (prolonged) blockade of the p53-dependent apoptotic response to genotoxic stress in the small intestine may influence tumor susceptibility, especially in backgrounds where small intestinal tumors occur such as in min mice or individuals with familial polyposis and especially if the p53 response is activated by various endogenous or exogenous exposures. Experimentally, this may be approached through either small intestine-specific deletion of p53 or through small intestine-specific expression of potent antiapoptotic genes. In summary, the present studies provide a foundation for future studies to analyze the genetic basis, therapeutic implications, and tissue specificity of the in vivo p53-mediated stress response.

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