p53-Dependent acinar cell apoptosis triggers epithelial proliferation in duct-ligated murine pancreas

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Scoggins, Charles R., Ingrid M. Meszoeyl, Michihiko Wada, Anna L. Means, Liying Yang, and Steven D. Leach. p53-Dependent acinar cell apoptosis triggers epithelial proliferation in duct-ligated murine pancreas. Am J Physiol Gastrointest Liver Physiol 279: G827–G836, 2000.—The mechanisms linking acinar cell apoptosis and ductal epithelial proliferation remain unknown. To determine the relationship between these events, pancreatic duct ligation (PDL) was performed on p53(+/+) and p53(−/−) mice. In mice bearing a wild-type p53 allele, PDL resulted in upregulation of p53 protein in both acinar cells and proliferating duct-like epithelium. In contrast, upregulation of Bel-2 occurred only in duct-like epithelium. Both p21WAF1/CIP1 and Bax were also upregulated in duct-ligated lobes. After PDL in p53(+/+) mice, acinar cells underwent widespread apoptosis, while duct-like epithelium underwent proliferative expansion. In the absence of p53, upregulation of p53 target genes and acinar cell apoptosis did not occur. The absence of acinar cell apoptosis in p53(−/−) mice also eliminated the proliferative response to duct ligation. These data demonstrate that PDL-induced acinar cell apoptosis is a p53-dependent event and suggest a direct link between acinar cell apoptosis and proliferation of duct-like epithelium in duct-ligated pancreas.

THE MAMMALIAN PANCREAS IS characterized by low resting rates of cell division but a significant proliferative response to mitogenic signals or tissue injury (11). Pancreatic epithelial proliferation may be initiated by soluble growth factors (16, 31, 40), inflammatory cytokines (12), islet cell injury (24), partial pancreatectomy (30), or pancreatic duct obstruction (1, 6, 33). After either islet cell injury or partial pancreatectomy, pancreatic regeneration appears to be initiated by proliferation of duct-like epithelial cells, with subsequent differentiation of new islet and/or acinar tissue (24, 30). Although controversial, this regenerative capacity has led Bouwens (3) and Sharma et al. (30) to propose a stem cell capacity within the adult pancreas.

In the case of pancreatic duct obstruction, proliferation is one component of a complex phenotype that involves a combination of cellular inflammation, acinar cell loss, generation of ductal-tubular complexes, and islet neogenesis (26, 34–36). Recent studies (36, 39) have demonstrated that pancreatic duct ligation (PDL) in mice or rats induces expression of multiple growth factors and cytokines, including gastrin, transforming growth factor-α (TGF-α), interleukin (IL)-1α, IL-1β, IL-6, IL-10, tumor necrosis factor-α, and Fas ligand. These events are associated with acinar cell apoptosis, which appears to represent the predominant mechanism of acinar cell loss after duct ligation (1, 6, 39). Coincident with the initiation of acinar cell apoptosis, significant proliferation is observed in duct-like epithelial cells, leading to the formation of ductal-tubular complexes with focal areas of islet neogenesis (2, 3, 35).

These lineage-specific changes in cell survival, proliferation, and differentiation induced by PDL provide the opportunity to gain important insight into factors regulating pancreatic stem cell biology (2, 3). Because of the complexity of histological events induced by duct ligation, previous studies have not evaluated whether duct-like epithelial proliferation represents a primary response to duct ligation or a secondary regenerative response to acinar cell loss. In addition, the molecular mechanisms by which duct ligation selectively induces apoptosis in acinar cells and proliferation in duct-like cells remain unknown.

In many cellular systems, initiation of apoptosis involves activation of the p53 tumor suppressor gene (5). p53 is a nuclear transcription factor known to transactivate several well-characterized target genes, including p21WAF1/CIP1, an inhibitor of cyclin-dependent kinase activity, and Bax, a positive regulator of apoptosis (8, 21). In the current study, we sought to determine the role of p53 in acinar cell apoptosis after PDL and also to evaluate the participation of candidate gene products known to mediate p53-dependent events. To facilitate genetic analysis, we refined a technique for PDL in the mouse and found that duct ligation stabilized p53 protein in both acinar and ductal cell lineages. p53 activation after duct ligation was further documented by increases in tissue expression of both p21WAF1/CIP1 and Bax. These events were associated with extensive

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acinar cell apoptosis, as evidenced by changes in cellular morphology and positive terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) labeling. In contrast to the widespread activation of p53 observed in both acinar and ductal lineages, upregulation of the antiapoptotic protein Bcl-2 was limited to highly proliferative duct-like epithelium. Using p53-null mice carrying homozygous deletions in the p53 allele [p53(−/−)], we found that the absence of p53 prevented not only the induction of apoptosis after PDL but also the associated proliferative response. These findings confirm that acinar cell apoptosis after duct ligation is a p53-dependent event and further suggest a link between acinar cell apoptosis and proliferation of duct-like epithelium.

METHODS

Mice, PDL, and tissue harvest. C57BL/6 × DBA male mice were utilized for both unoperated controls and PDL in the setting of wild-type p53 [p53(+/+)]. All animals were fed standard murine chow and water ad libitum. Twelve mice underwent PDL in the following manner. After intraperitoneal injection with a mixture of ketamine (80 mg/kg) and xylazine (20 mg/kg), the peritoneum was entered through a midline laparotomy and the stomach, pancreas, and spleen were mobilized. The spleen was retracted laterally, and the splenic vessels were separated from the pancreatic tissue to prevent devascularization of pancreatic parenchyma. The splenic lobe of the pancreas was ligated to the left of the portal vein with a 5-0 monofilament polypropylene suture. The viscera were replaced in anatomic position, and the incision was closed in two layers using 4-0 braided polyglycolic acid suture. A subcutaneous injection of 1 ml sterile saline solution was given at the conclusion of the procedure. To examine the effects of PDL in the absence of p53, C57BL/6J-Trp53tm1Tyj [p53(−/−)] male and C57BL/6J-Trp53tm1Tyj [p53(−/−)] female mice were obtained from Jackson Laboratories (Bar Harbor, ME). Offspring from the p53(−/−) × p53(−/−) cross were genotyped by PCR as previously described (18), and homozygous p53(−/−) mice (n = 10) were subjected to PDL as described above.

Mice were killed 1, 3, and 5 days after PDL. For studies of cellular proliferation, bromodeoxyuridine (BrdU) (75 mg/kg) was injected intraperitoneally 2 h before death. Unoperated and duct-ligated mice were killed, and pancreatic tissues were harvested. The splenic lobes were fixed in 4% paraformaldehyde for 3 h at 4°C then washed and stored in 70% ethanol at 4°C overnight. Tissues were then embedded in paraffin and sectioned at 5 μm. In a subset of mice, a segment of the splenic lobe was reserved for Western blot analysis before tissue fixation.

Immunostaining. Immunohistochemical analysis was performed in the following manner. Sections (5 μm) were fixed to APS-coated glass slides, dewaxed, and rehydrated. After quenching of endogenous peroxidase activity using 3% H2O2 for 5 min, the slides were blocked in 5% normal serum for 30 min at room temperature. The slides were then treated with primary antibody overnight at 4°C. Primary antibodies and dilutions were as follows: rabbit anti-amyrase, 1:1,000 (Sigma Chemical, St. Louis, MO); anti-BrdU, prediluted (Zymed Laboratory, San Francisco, CA); mouse anti-Bcl-2 clone 124, 1:100 (Dako); and rabbit anti-p53 CM5, 1:1,000 (Novo Castra, Burlingame, CA). After washing in PBS, species-specific biotinylated secondary antibody (Vector Laboratories, Burlingame, CA) was applied for 60 min at room temperature and followed by application of avidin-biotin complex-conjugated solution (Vector Laboratories) for 30 min at room temperature. 3,3'-Diaminobenzidine (DAB) chromogen was applied, and the slides were counterstained with hematoxylin.

Conjugated lectin staining was performed on paraaffin-embedded sections in the following manner: after dewaxing and rehydrating, the tissues were permeabilized with 0.2% Triton X-100/PBS for 10 min, then blocked with 2% BSA solution for 1 h. As a marker for acinar cell apical membranes, rhodamine-conjugated peanut agglutinin (PNA) (Pierce, Rockford, IL) was applied for 1 h at room temperature (1:100, Vector Laboratories). After PBS washes, nuclei were counterstained with YO-PRO-1 for 5 min (1:50,000, Molecular Probes, Eugene, OR) and mounted with GVA mounting solution (Zymed Laboratory). As a structural marker of ductal epithelium, fluorescent staining was performed on frozen sections with rhodamine-conjugated dolichos biflorus agglutinin (DBA) (1:100, Vector Laboratories) as described above.

Immunohistochemical analysis for apoptosis was performed with the Apoptag kit (Intergen, Purchase, NY). The slides were dewaxed and rehydrated, then treated with proteinase K (5 μl of 20 mg/ml stock solution in 5 ml PBS) for 15 min at room temperature. After quenching in 3% H2O2 for 5 min, the equilibration buffer was applied for 30 s, followed by treatment with the terminal deoxynucleotidyl transferase enzyme for 1 h at 37°C. Stop buffer was applied, and slides were washed. Anti-digoxigenin-peroxidase was applied for 30 min. The slides were then treated with DAB solution for 5 min and counterstained with methyl green. The slides were dehydrated, and coverslips were applied.

Cell counts and statistical analysis. To obtain quantitative data regarding the rate of ductal-tubular complex formation in ligated pancreatic lobes, duct-like structures from control, p53(+/+) PDL, and p53(−/−) PDL lobes were counted under light microscopy. Means ± SE were obtained, and unpaired t-tests were performed comparing the number of small ducts per low-power field (>200) in both PDL and control tissues. To obtain quantitative data for BrdU incorporation and TUNEL positivity, cells were counted under ×200 light microscopic magnification. Means ± SE were obtained, and unpaired t-tests were performed. Statistical analysis was performed with Prism (GraphPad Software, San Diego, CA).

Protein extraction and Western blotting. Proteins were extracted from minced pancreatic tissues by solubilizing in lysis buffer [50 mM Tris, pH 7.5, 100 mM NaCl, and 0.5% Nonidet P-40 supplemented with protease inhibitors (chymostatin, leupeptin, antipain, and pepstatin)] on a rotating platform at 4°C for 1 h. Insoluble debris was pelleted, and the protein concentration of the resulting supernatant was determined using the Bradford method. Fifty micrograms of total protein per lane were loaded and resolved on SDS-10% polyacrylamide gels, transferred to polyvinylidene difluoride membrane (Immobilon-P, Millipore), and probed with the following primary antibodies from Santa Cruz Biotechnology (Santa Cruz, CA): mouse monoclonal anti-p53 antibody (DO-1, 1:500); rabbit polyclonal anti-Bax antibody (P-19, 1:1,000); and rabbit polyclonal anti-p21 antibody (1:1,000). The membranes were washed and incubated with horseradish peroxidase-conjugated species-appropriate secondary antibodies (Pierce, Rockford, IL), then developed with enhanced chemiluminescence reagents (Amersham Life Science, Little Chalfont, UK) and exposed to radiograph film.

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RESULTS

Acinar cell loss and expansion of duct-like epithelium after PDL. Murine PDL resulted in phenotypic changes in a time-dependent manner (Fig. 1). Three days after PDL, the ligated lobe was edematous, and there was a marked inflammatory cell infiltrate, consisting mostly of segmented neutrophils. The acinar architecture was poorly organized, with loss of nuclear polarity and irregular acinar borders. Apoptotic bodies were visible in some acinar units. The total volume of acini appeared to be reduced, and there was expansion of a novel duct-like epithelium composed of low-cuboidal cells. The islets appeared to be unaffected by PDL. By 5 days after PDL, there were few remaining acini, and these appeared to be poorly organized. The gland was fibrotic and showed an extensive inflammatory process composed predominantly of mononuclear cells. There were numerous duct-like structures throughout the ligated lobe.

As a structural marker of ductal epithelium, DBA lectin staining was performed on frozen sections of control and duct-ligated pancreas. Control pancreatic tissues showed strong staining in large, interlobar-type ducts as well as small intercalated ducts. The staining pattern primarily involved cytoplasmic membrane, with less intense cytoplasmic staining. DBA staining of acini, islets, and blood vessels was not observed (Fig. 1D). Five days after PDL, large interlobar ducts continued to be stained by DBA. The novel duct-like epithelium also stained positively for DBA, indicating expression of mature ductal membrane carbohydrate moieties. As in the case of control pancreas, nonductal elements showed no evidence of DBA staining (Fig. 1, E and F).

Lineage-specific analysis of p53 and Bcl-2 expression after PDL. To determine the molecular mechanisms responsible for the divergent behavior of acinar and ductal lineages after PDL, we analyzed lineage-specific expression of Bcl-2 and p53 using immunohistochemistry on control and duct-ligated pancreas. Control pancreatic tissues did not stain positively for Bcl-2 (Fig. 1G). However, 3 days after PDL, the newly emerging duct-like epithelium showed positive supranuclear/cytoplasmic staining for Bcl-2. Some mononuclear cells were also Bcl-2 positive. In contrast, acinar cells showed no evidence of Bcl-2 expression after duct ligation (Fig. 1H). Five days after PDL, pancreatic tissue showed ongoing strong expression of Bcl-2 within the duct-like epithelium. The few acinar cells remaining at this point did not express Bcl-2, consistent with widespread induction of apoptosis in this cell type (Fig. 1I). Immunohistochemical analysis for p53 in control pancreas demonstrated little-to-no detectable p53 protein in acinar cell, ductal, or islet nuclei (Fig. 1D). Five days after PDL, however, there was strong nuclear positivity in nuclei of both acinar and duct-like cells, indicating stabilization of p53 protein (Fig. 1, K and L).

PDL induces p53-dependent activation of Bax and p21WAF1/CIP1. Based on the uniform stabilization of p53 observed in both acinar and ductal lineages after PDL, we next examined tissue expression of Bax and p21WAF1/CIP1, two well-characterized p53 target genes (8, 21). To confirm p53-dependent transactivation of target genes, pancreatic tissue was harvested from duct-ligated p53(+/+) and p53(−/−) mice 5 days postligation, and protein lysates were analyzed by Western blot. Pancreatic tissue from p53(+/+) control mice showed low levels of detectable p53 protein. Five days after PDL, pancreatic tissue from p53(+/+) mice demonstrated significant upregulation of p53 protein, consistent with the immunohistochemical staining pattern described above. As expected, no evidence of p53 protein expression was observed in tissue harvested from p53(−/−) mice, even after duct ligation (Fig. 2). Western blot analysis of Bax expression in p53(+/+) mice showed low levels of Bax protein in control pancreas but significant upregulation 5 days after PDL. No evidence of Bax upregulation was observed after PDL in p53(−/−) mice, confirming the p53-dependent nature of this effect (Fig. 2B). Similarly, analysis of p21WAF1/CIP1 protein in p53(+/+) mice demonstrated minimal expression in control pancreas, but significant upregulation 5 days after duct ligation. No upregulation of p21WAF1/CIP1 protein was observed after duct ligation in p53(−/−) mice (Fig. 2B). These results demonstrate induction of two known p53 target genes in pancreatic tissue after duct ligation, confirming functional activation of p53 protein.

PDL-induced acinar cell apoptosis is a p53-dependent event. Based on the upregulation of p53 protein and transactivation of p53 target genes observed after PDL in p53(+/+) mice, we next sought to determine whether PDL-induced acinar cell apoptosis represented a p53-dependent event. The requirement for p53 in PDL-induced acinar cell apoptosis was rigorously assessed by comparison of acinar cell markers and TUNEL staining in control lobes, p53(+/+) ligated lobes, and p53(−/−) ligated lobes. Histological examination of p53(−/−) pancreatic tissue 5 days after duct ligation revealed fibrosis, inflammatory cells, and interstitial edema throughout the ligated lobe, but preservation of acinar cells. The acini were distorted, but the nuclei retained their basal polarity compared with pancreatic tissue from duct-ligated p53(+/+) mice (Fig. 3, C and B, respectively). Preservation of acinar cell mass after duct ligation in p53(−/−) mice, but not p53(+/+) mice, was confirmed using both immunostaining for amylase and Cy3-conjugated PNA lectin staining to mark acinar cell membranes. Control acini stained positive for amylase, whereas ducts and islets did not (Fig. 3D). Five days after duct ligation, there were only rare remaining amylase-positive cells in pancreatic tissue from p53(+/+) mice (Fig. 3E). In contrast, the pancreatic tissue from duct-ligated p53(−/−) mice retained amylase immunoreactivity within preserved acini, confirming preservation of viable acinar cell mass (Fig. 3F). PNA labeling of control pancreatic tissues revealed specific staining of acinar cell apical membranes, with no labeling of ductal, vascular, or islet tissues (Fig. 3G). In contrast, pancreatic tissue from duct-ligated p53(+/+) failed to label with
Fig. 1. Phenotypic changes after pancreatic duct ligation (PDL). A: normal appearance of unoperated control mouse pancreas, showing preponderance of acinar cells, with islets and ducts constituting a minority of cellular mass. *, Duct; I, islet; A, acinus. B: representative section of mouse pancreas 3 days after PDL. Note inflammation, fibrosis, loss of acinar architecture, and emergence of numerous small duct-like structures (arrow). C: representative section 5 days after PDL. Pancreas is fibrotic and inflamed, with few remaining acini. Note small duct-like structures lined by low cuboidal epithelium (arrow). D: *dolichos biflorus* agglutinin (DBA) staining of control pancreas. Both interlobular and intercalated ducts label with DBA (red fluorescence). Nonductal tissue labels only with Yo-Pro-1 green counterstain. E–F: DBA staining of mouse pancreas 5 days after PDL. The emerging duct-like epithelium is labeled by DBA. G–J: Bcl-2 immunohistochemistry in control and duct-ligated pancreatic tissues. G: absence of Bcl-2 staining in control pancreatic tissues. H: 3 days after PDL, duct-like epithelium expresses Bcl-2 (arrow), whereas acini are Bcl-2 negative. I: 5 days after PDL, duct-like epithelium continues to express Bcl-2. Bracket indicates Bcl-2-positive intercalated ductal epithelium. Note negative staining in adjacent acinar cells (*). J–L: p53 immunohistochemistry in control and duct-ligated pancreas. J: control pancreas showing no p53-positive nuclei. K: duct-ligated pancreas 5 days after PDL, p53-positive nuclei are evident in both duct-like epithelium and few remaining acini. L: higher magnification view demonstrating p53-positive nuclei in both acinar cells and duct-like epithelium 5 days after PDL. Arrows, p53-positive acinar cell nuclei; arrowhead, p53-positive duct cell nucleus. Original magnifications: A–E, ×200; F–H, J, and K, ×400; I and L, ×1,000. Counterstaining: A–C, hematoxylin and eosin; D–F, Yo-Pro-1; G–L, hematoxylin.
Fig. 2. Analysis of p53 target genes in p53(+/-) and p53(-/-) mice. A: PCR genotyping demonstrates p53(+/-), p53(+/-), and p53(-/-) null mice: 1st and 2nd lanes, p53(+/-) mouse; 3rd and 4th lanes, p53(+/-) heterozygous mouse; 5th and 6th lanes, p53(-/-) homozygous null mouse. B: Western blot analysis of protein lysates from control, p53(+/-) PDL day 5, and p53(-/-) PDL day 5 tissues. Top, p53 protein in pancreatic tissue. Note low level of p53 expression in control tissues and upregulation in p53(+/-) PDL pancreatic tissue 5 days after duct ligation. No p53 protein is detected in p53(-/-) pancreas. Middle, Bax expression in same lysates. Weak expression is noted in control tissues. Note Bax upregulation in p53(+/-) PDL condition, with no increase observed in p53(-/-) PDL pancreatic tissue. Bottom, p21WAF1CIP1 expression. Note p21WAF1CIP1 upregulation in p53(+/-) PDL condition. No p21WAF1CIP1 protein is detected in p53(-/-) PDL pancreatic tissue: 1st lane, p53(+/-) unoperated control pancreas; 2nd lane, p53(+/+) pancreas 5 days after duct ligation; 3rd lane, p53(-/-) pancreas 5 days after duct ligation.

PNA, confirming near total loss of acinar cells (Fig. 3H). In contrast, pancreatic tissue from duct-ligated p53(-/-) mice retained PNA-positive acini, suggesting retention of acinar apical membrane integrity in the absence of p53.

To assess the rate of apoptosis induced by PDL, TUNEL labeling of apoptotic DNA fragments was performed on pancreatic tissue from control, duct-ligated p53(+/-) and duct-ligated p53(-/-) mice. Control tissue demonstrated no specific TUNEL staining (Fig. 3J), whereas pancreatic tissue from duct-ligated p53(+/-) mice had numerous TUNEL-positive apoptotic nuclear bodies throughout the ligated lobe. TUNEL-positive nuclear bodies were primarily located outside of duct-like epithelium, suggesting selective apoptosis of acinar cells (Fig. 3K). In contrast, PDL in p53(-/-) mice was not associated with a large increase in TUNEL-positive nuclear staining. There was only rare staining observed in acinar, ductal, or islet cell populations, suggesting failure to induce apoptosis in the absence of functional p53 (Fig. 3L). Quantification of TUNEL-positive nuclei revealed no positively labeled nuclei in the control tissue, 161.3 ± 29.8 positive nuclei per ×200 field in pancreatic tissue from duct-ligated p53(+/-) mice, and 0.25 ± 0.25 positive nuclei in pancreatic tissue from duct-ligated p53(-/-) mice (Table 1). These results demonstrate that PDL-induced acinar cell loss is primarily mediated by apoptosis and that this response requires functional p53 protein.

With respect to the inflammatory response associated with PDL, the cellular inflammatory infiltrate appeared to be somewhat attenuated but not eliminated in duct-ligated p53(-/-) pancreas compared with duct-ligated p53(+/-) controls. This change consisted primarily of a reduction in the number of inflammatory cells rather than a change in character of the inflammatory infiltrate. The magnitude of edema and fibrosis appeared unchanged. This specific reduction in cellular inflammation may be due to the preservation of acinar cells in the absence of p53, suggesting that a component of the inflammatory infiltrate is specifically recruited in response to apoptotic cellular debris. However, the significant amount of edema and inflammation observed in the absence of p53 suggests that both apoptotic and nonapoptotic events contribute to the inflammatory response initiated by duct ligation.

PDL-induced epithelial proliferation does not occur in the absence of acinar cell apoptosis. The combination of acinar cell apoptosis and expansion of a novel duct-like epithelium observed after PDL raises the question of whether proliferation of duct-like epithelium represents a primary response to duct ligation or, alternatively, a regenerative response to acinar cell apoptosis. To address this question, we specifically analyzed epithelial proliferation in duct-ligated p53(+/-) and p53(-/-) mice. Five days after PDL, p53(+/-) tissues demonstrated emergence of a novel duct-like epithelium (Fig. 3B) while pancreatic tissue from p53(-/-) mice displayed an attenuated number of duct-like structures (Fig. 3C). Quantification of duct-like structures per ×200 field revealed a mean of 9.8 ± 0.8 in pancreatic tissue from control mice, 123.5 ± 3.9 in duct-ligated p53(+/-) pancreas (P < 0.0001 vs. control and p53(-/-) PDL), and 20.8 ± 1.8 in duct-ligated p53(-/-) pancreatic tissue (P < 0.0001 vs. control) (Table 1). These results demonstrate an attenuated expansion of duct-like epithelium in the absence of acinar cell apoptosis.

As a marker of cellular proliferation, BrdU incorporation was assessed via immunohistochemistry. Control tissues incorporated BrdU at low frequency, with rare acinar cell and occasional ductal cell nuclear positivity (Fig. 4A). In contrast, duct-ligated p53(+/-) pancreatic tissue incorporated BrdU at a high rate, primarily within newly formed duct-like epithelium. The few remaining acinar cells did not show evidence of BrdU incorporation (Fig. 4B). In contrast to the high rate of epithelial proliferation observed after duct ligation in p53(+/-) mice, tissue from duct-ligated
p53(-/-) mice showed only occasional BrdU-positive nuclei, primarily among interstitial cells (Fig. 4C). Quantification of BrdU-positive nuclei per ×200 field revealed a mean of 12.5 ± 1.3 labeled nuclei in control tissues, 63.8 ± 4.9 nuclei in duct-ligated p53(+/+) pancreas (P < 0.0001 vs. control), and 13.2 ± 0.9 labeled nuclei in pancreatic tissue from duct-ligated p53 -/- mice (P = nonsignificant vs. control) (Table 2).
TABLE 1. Rate of small duct formation 5 days after PDL in p53(+/+ ) and p53(−/− ) mice

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<tr>
<th>Ducts/LPP</th>
<th>p53(+/+) Control</th>
<th>p53(+/+) PDL</th>
<th>p53(−/−) PDL</th>
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<td>9.8 ± 0.8 n</td>
<td>123.3 ± 3.9 †</td>
<td>20.8 ± 1.8 †</td>
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Values for ducts/low-power magnification field (LPP; ×200) are means ± SE. PDL, pancreatic duct ligation; p53(+/+), wild-type mice; p53(−/−), knockout mice. *P < 0.0001 vs. p53(−/−) PDL animals by unpaired t-test; †P < 0.0001 vs. p53(+/+) control animals.

The current results confirm earlier reports indicating that acinar cell apoptosis represents the dominant mechanism of acinar cell death after PDL (1, 6, 13). Prior studies (13, 28) have suggested species-to-species variability in the relative contributions of apoptotic and necrotic acinar cell death after PDL, with apoptosis predominating in the rat, while necrosis predominates in the opossum. In the mouse, selective PDL is technically difficult, resulting in a limited number of studies utilizing this species. Watanabe and colleagues (37) initially reported the successful application of splenic lobe duct ligation in the mouse, resulting in loss of acinar cell mass and ductal proliferation similar to that observed in the rat. Two subsequent studies (1, 39) have documented acinar cell apoptosis as the predominant mechanism of acinar cell death after PDL in the mouse. In one of these studies (39), murine PDL was associated with increases in expression of Fas ligand and IL-1β-converting enzyme as assessed by RT-PCR. However, the precise mechanism by which acinar cell apoptosis is initiated after PDL has not previously been elucidated. Our data suggest induction of a classical p53-dependent apoptotic pathway involving stabilization of wild-type p53 protein and transactivation of p53 target genes, including p21WAF/CIP and Bax. Both the p21WAF/CIP and Bax genes contain consensus p53-binding sites in their regulatory elements (8, 21). p53 is also known to increase expression of Fas receptor (4, 25), suggesting relevance between our findings and those of Yasuda et al. (39). With respect to the ability of Fas ligand and other signals to potentially induce apoptosis in a p53-independent manner (7), the present results strictly define PDL-induced acinar cell apoptosis as a p53-dependent event.

The present results also provide insight regarding the expansion of duct-like epithelium observed after PDL. This epithelium has been histologically well characterized in previous studies (34, 35, 37). In the rat, PDL induces expansion of ductular-tubular complexes characterized by high rates of cellular proliferation (33–35). Evidence of ductal differentiation has previously been suggested based on positive immunohistochemical staining for cytokeratin 20 (35). However, additional studies (3, 35) have demonstrated similarities with embryonic pancreatic epithelium, including expression of the glut-2 glucose transporter protein and initiation of islet neogenesis. Bertelli and Bendayan (2) have reported the appearance of intermediate cells displaying features of both endocrine and exocrine differentiation after PDL in the rat. In this regard, the expanding ductal epithelium induced by PDL shares features in common with several other models of pancreatic epithelial proliferation, including islet cell injury (24), transgenic overexpression of interferon-γ (12), partial pancreatectomy (30), and transgenic overexpression of TGF-α (31).
In each of these models, an initiating stimulus results in loss of a differentiated cell population, suggesting that associated epithelial proliferation may represent a compensatory, regenerative response. However, it remains unclear whether the observed proliferation is directly triggered by differentiated cell loss as opposed to a direct effect of the initiating stimulus. In the present study, the absence of acinar cell apoptosis after PDL in p53(-/-) mice represents a unique opportunity to directly study the relationship between acinar cell apoptosis and associated proliferation of duct-like epithelium. Specifically, is proliferation in this model a direct effect of PDL or a regenerative response initiated by loss of acinar cells? Our results show that

Table 2. Quantification of BrdU incorporation and TUNEL assay positivity

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<th>p53 (+/+)/Control</th>
<th>p53 (+/+) PDL</th>
<th>p53 (-/-) PDL</th>
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<tr>
<td>BrdU incorporation</td>
<td>12.5 ± 1.3</td>
<td>63.8 ± 4.9*</td>
<td>13.2 ± 0.9</td>
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<tr>
<td>TUNEL assay</td>
<td>0.0</td>
<td>161.3 ± 29.8†</td>
<td>0.25 ± 0.25</td>
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Values are means ± SE. Bromodeoxyuridine (BrdU) incorporation given as no. of positive ductal epithelial nuclei/×200 field. Terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) assay given as no. of positive acinar nuclei/×200 field.

*P < 0.0001 vs. p53(+/+) control and p53(-/-) PDL animals by unpaired t-test. †P < 0.01 vs. p53(+/+) control and p53(-/-) PDL animals by unpaired t-test. Values for p53(-/-) PDL animals are nonsignificant vs. control animals.
PDL-induced ductal proliferation does not occur in the absence of acinar cell apoptosis, consistent with the view that ductal proliferation represents a regenerative response to acinar cell loss.

This system also provides insight into factors determining lineage-specific influences on cell survival in pancreatic tissue. While our results do not directly identify the mechanism responsible for differential survival of acinar and ductal cells following PDL, selective upregulation of Bcl-2 in duct-like epithelium may act as an important survival signal. Bcl-2 acts as a regulator of mitochondrial membrane potential and is capable of preventing apoptosis induced by multiple stimuli, including direct activation of p53 (14, 19, 27). We have observed Bcl-2 upregulation in proliferating epithelium beginning on day 3 after PDL, with ongoing expression on day 5. During this time period, acinar cells show no detectable evidence of Bcl-2 expression and undergo widespread apoptosis. Similar results have been reported in the rat, in which increased Bcl-2 expression was noted as early as 6 h after PDL. Based on our additional observations suggesting activation of p53 in both acinar and ductal lineages, these findings suggest that Bcl-2 may exert an important survival influence for cells in the proliferating duct-like epithelium.

In several ways, the current results suggest analogy between the epithelial response to PDL and the hepatic epithelial response to bile duct ligation. Bile duct ligation in the mouse results in widespread apoptosis among hepatocytes, accompanied by proliferation of duct-like biliary epithelium (22–23). In this system, hepatocyte apoptosis is apparently mediated by both Fas-dependent and Fas-independent pathways (22). Changes in the expression of both Bcl-2 and Bax have been also reported (17, 32), although the requirement for p53 remains unknown. As in the case of PDL, bile duct ligation in the rat results in increased expression of specific growth factors, including TGF-α (23). These and other findings (17, 22, 23, 32) have led to the suggestion that bile duct ligation induces expansion of a stem cell compartment.

In this regard, expansion of Bcl-2-expressing epithelium appears to represent a common component of epithelial regeneration and repair (10, 29). During embryonic development, Bcl-2 expression is frequently noted in cells undergoing morphological transition from undifferentiated stem cells to committed precursor cells (20). In the adult, Bcl-2 expression in many tissues is topographically restricted to progenitor cell zones, including the basal layer of skin, basal cells of prostate epithelium, and the lower half of crypts in small and large intestine (15, 20). It is, therefore, possible that the Bcl-2-expressing duct-like epithelium observed after PDL may represent a cell population with progenitor function. This interpretation is consistent with previous studies (3, 35) demonstrating similarities between these ductal complexes and embryonic epithelium.

In summary, we have demonstrated that murine PDL induces widespread activation of p53 and trans-activation of p53 target genes in pancreatic tissue. This response is associated with extensive acinar cell apoptosis and proliferation of duct-like epithelial cells expressing high levels of Bcl-2. Acinar cell apoptosis in this system represents a p53-dependent event, and proliferation of duct-like epithelium is not observed in the absence of acinar cell apoptosis. These findings are consistent with induction of a regenerative response that may involve expansion of cells with progenitor function.

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