Role of p53-Dependent Activation of Caspases in Chronic Obstructive Uropathy: Evidence from p53 Null Mutant Mice

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Abstract. Chronic obstructive uropathy (COU) created by unilateral ureteric ligation is associated with increased renal cell apoptosis and p53 expression. Genetically engineered mice were used to examine the role of p53 in renal cell apoptosis in COU and the involved molecular pathways. Obstructed kidneys in p53+/+ and p53−/− mice were examined at days 4, 7, 15, 20, and 30 for apoptosis, and mRNA were examined for p53, members of the bcl-2 family, the death receptor family, and the common effectors of apoptosis. Obstructed kidneys in p53+/+ mice displayed upregulation of mRNA for members of the bcl-2 family and most of the death receptor family, except for a lower level of tumor necrosis factor receptor-1, TRAIL, and FAP in p53+/+ mice. Obstructed kidneys in p53+/+ and p53+/− mice showed virtual absence of caspase 11 and marked attenuation of caspases 1 and 12, contrasted with their strong expression in p53+/+ kidneys. These data suggest that apoptosis in obstructed kidneys involves p53-dependent as well as p53-independent pathways. The p53-dependent pathway may involve activation of caspases 1, 11, and 12, whereas the p53-independent pathway may involve activation of members of the bcl-2 and death receptor families.

Kidneys with chronic obstructive uropathy (COU) develop progressive tubulointerstitial damage (1–3). Although several tubular changes, such as dilation, atrophy, and immature phenotype, are widely recognized, tubular cell apoptosis has emerged recently as a crucial lesion in COU (1,3,4). We demonstrated a marked increase in tubular and interstitial cell apoptosis in kidneys with COU (3), and this increase is associated with a parallel increase in p53 mRNA. Increased p53 expression during apoptosis has been reported in cells of diverse origins, including hepatocytes (5) and neurons (6), and in cultured cells treated with DNA-damaging agents (7,8). These observations suggest that p53 activation may have a role in the development of apoptosis after ureteric obstruction.

The molecular control of apoptosis is only partially understood. It likely involves two distinct pathways that share a common downstream arm. The first pathway involves the activation of death receptors (Fas, tumor necrosis factor-1 [TNFR-1], TRAIL) by their respective ligands (9–13). The generated signal activates cytosolic adapter molecules (e.g., TRADD, RIP, FADD, FAF) and results in the conversion of pro-caspase 8 to caspase 8. Caspase 8 converts pro-caspases 3, 6, and 7 into their respective active caspases, which are responsible for the cellular changes that are characteristic of apoptosis (10,11,14). The second pathway involves the bcl-2 family and is initiated by a large variety of environmental stresses, such as radiation, heat, or growth factor deficiency (15–18). These signals are transmitted to the mitochondria by an unknown mechanism and influence the balance between apoptosis promoters (bax, bcl-xS, bad, and bak) and inhibitors (bcl-2, bcl-xL, bcl-w, and bfl-1) among the bcl-2 family. These proteins reside in the mitochondrial membrane and share structural homology (15,16). Apoptotic signals that originate from this pathway result in the activation of pro-caspase 9 by cytochrome c and apoptosis protease activating factor-1 (15,19). Caspase 9, in turn, activates pro-caspases 3, 6, and 7, a step that represents the onset of the common arm of both death receptor and bcl-2 apoptotic pathways (15). Other caspases, including caspases 1, 11, and 12, have been recognized recently (20) and reported to have a regulatory role in apoptosis and inflammatory response (20–22). However, their location in the apoptotic pathways has not been firmly established.

Although it is well established that p53 promotes apoptosis...
(6,23–25), the underlying molecular mechanism for its action remains unclear. Although interaction between p53 protein and the death receptor pathway has not been established, p53 can regulate the cellular levels of members of the bcl-2 family, including bax and bcl-2 (23,26,27). In addition, p53 has been suggested recently to activate a number of caspases in vitro (28,29). Last, p53 can induce cell-cycle arrest through upregulation of the cyclin-dependent kinase inhibitor p21Waf1/Cip1 (23,30,31).

In this study, we report that p53 gene deficiency attenuates renal cell apoptosis after ureteric obstruction. This effect may be attributable to downregulation of caspase activity, including caspases 1, 11, and 12.

**Materials and Methods**

**p53-Deficient Mice**

p53-deficient mice, generated by disruption of exon 5 of the p53 gene in murine embryonic stem cells (32), were generously provided by Dr. Allan Bradley (Institute for Molecular Genetics, Baylor College of Medicine, Houston, TX). The mice used in this study had mixed genetic backgrounds (approximately 75% from C57BL/6 and approximately 25% from 129/Sv strain). Neither p53 mRNA nor p53 protein was expressed in tissues, including kidneys, from these null mutant mice. Because p53 null (−/−) mice are sterile, p53−/− mutant mice. Because p53 null (−/−) mice are sterile, p53−/− mice also should have displayed light microscopic features of apoptosis (shrunken or fragmented and condensed nuclei). Unequivocally positive cells without obvious light microscopic features of apoptosis were also counted because it has been shown that they may represent cells in which RNA fragmentation already occurs but precedes other apoptotic changes that are recognizable by light microscopy (3). Necrotic cells, which were stained positive, were not counted. These cells occurred in small patches involving several contiguous cells at the papillary tip and were distinguished easily from apoptotic cells, which occurred in isolation and were scattered throughout the renal parenchyma. Necrosis occurred in three obstructed kidneys only and was not a significant lesion after ureteric obstruction. Apoptotic cells were counted under the 40× objective of a Nikon microscope. All apoptotic cells within 5 to 10 random fields were counted, each delineated by a 1-cm2 ocular grid attached to the eyepiece. Differentiation between cortex and medulla regions in obstructed kidneys was possible until 20 d postobstruction, and the random fields chosen in the same manner. It should be noted that interstitial and inflammatory cells appear similar as they develop apoptosis; hence, precise quantification of interstitial cell apoptosis was performed in the same manner. It should be noted that interstitial and inflammatory cells appear similar as they develop apoptosis; hence, precise quantification of interstitial and inflammatory cells that were undergoing apoptosis was not carried out.

**DNA Extraction and Southern Blotting**

To determine p53 genotypes of the bred mice, DNA was extracted from tail fragments, as previously described (32). Ten μg of genomic DNA from each animal were cleaved with BamHI, subjected to 0.9% agarose gel electrophoresis, blotted onto nylon membrane, hybridized to a probe specific for exons 2 through 6 of the p53 gene (32), washed, and autoradiographed. This probe detects both the wild-type and mutant p53 genes. However, because of the deletion of exon 5 in p53 null mice, the DNA product detected by the probe in p53 null mice is smaller than that seen in wild-type samples and, thus, easily distinguishable.

**Creation of COU**

Under inhalation anesthesia with methoxyflurane, COU was created in 12-wk-old, p53+/+; p53+/-, and p53−/− mice by complete ligation of the left ureter at the ureteropelvic junction with the use of double silk sutures. Animals were placed on regular diets, allowed free access to tap water, and killed at day 4, 7, 15, 20, or 30. Three to six mice were used for each time point. These time points were chosen because, in a pilot study, they were shown to span the entire quantitative spectrum of tubular and interstitial cell apoptosis. Kidneys of p53+/+; p53+/-, and p53−/− sham-operated mice were harvested at days 0, 15, and 30 to confirm that mice without urinary obstruction of these genotypes have the same pattern of apoptosis.

**Tissue Preparation**

Control, ligated, and contralateral kidneys were harvested. Portions of the kidney tissue were fixed in 10% formalin for in situ end labeling of fragmented DNA and routine histology (hematoxylin and eosin, periodic acid-Schiff, and trichrome stains). Tissue portions were also snap-frozen in liquid nitrogen and stored at −70°C for subsequent RNA extraction. Because the obstructed kidneys of the same genotype (see Results section), kidney tissues from mice of the same genotype, obtained at the same experimental time points, were pooled for RNA extraction.

**Light Microscopy and Morphometry**

Tissue sections were observed independently by two renal pathologists (Y.-J.C. and L.D.T.) who were unfamiliar with the experimental conditions. Morphometric evaluation of renal tubular diameters and interstitial volume was assessed as described previously (3). Briefly, a graded 1-cm micrometer, viewed under the 40× objective of a Nikon microscope (Melville, NY), was used to measure tubular diameters in periodic acid-Schiff–stained sections. The diameter (in micrometers) was measured for 20 random tubular cross sections in both the cortex and the medulla in each kidney section. Interstitial volume was determined with the use of a point-counting technique on trichrome-stained sections. The interstitial volume was expressed as the percentage of grid points of a 1-cm2 graded ocular grid, which is situated within the interstitial area, viewed at 20× magnification. Five to 10 random fields were used for morphometry.

**Detection and Quantification of Renal Cell Apoptosis by In Situ End Labeling of Fragmented DNA**

In situ end labeling of fragmented DNA was performed as detailed previously (3). The following morphologic criteria were used to ensure accurate identification of apoptotic cells: Only tissue sections without background staining were used for evaluation. Most positive cells also should have displayed light microscopic features of apoptosis (shrunken or fragmented and condensed nuclei). Unequivocally positive cells without obvious light microscopic features of apoptosis were also counted because it has been shown that they may represent cells in which RNA fragmentation already occurs but precedes other apoptotic changes that are recognizable by light microscopy (3). Necrotic cells, which were stained positive, were not counted. These cells occurred in small patches involving several contiguous cells at the papillary tip and were distinguished easily from apoptotic cells, which occurred in isolation and were scattered throughout the renal parenchyma. Necrosis occurred in three obstructed kidneys only and was not a significant lesion after ureteric obstruction. Apoptotic cells were counted under the 40× objective lens of a Nikon microscope. All apoptotic cells within 5 to 10 random fields were counted, each delineated by a 1-cm2 ocular grid attached to the eyepiece. Differentiation between cortex and medulla regions in obstructed kidneys was possible until 20 d postobstruction, and the random fields chosen in the same manner. It should be noted that interstitial and inflammatory cells appear similar as they develop apoptosis; hence, precise quantification of interstitial and inflammatory cells that were undergoing apoptosis was not carried out.

**Northern Hybridization for p53 mRNA**

Northern hybridization was used to determine p53 gene expression in control, contralateral, and ligated kidneys of mice that belonged to the same genotype (p53+/+; p53+/-, and p53−/−). Pooled kidney tissues, at each time point, were used to extract total cellular RNA, with the use of the RNAsol-B method (Tel Test B, Friendswood, TX).
Ten μg of the total RNA were loaded per lane onto 1% denaturing agarose gel and separated by electrophoresis. RNA was transferred to a nylon membrane and hybridized simultaneously with 32P-labeled cDNA probes for p53 mRNA (32) and 28S rRNA, respectively.

RNase Protection Assay

RNase protection assay was carried out per the manufacturer’s instructions (RiboQuant kit; PharMingen, San Diego, CA) and used to detect and quantify the expression of mRNA of apoptosis-related molecules (33,34). Three sets of template cDNA were used, each containing multiple cDNA probes, which allowed simultaneous detection of mRNA corresponding to the death receptor family (Fas, Fas ligand, TNFR-1, TRAIL, FADD, TRADD, RIP, FAP, and FAF), bcl-2 family (bax, bad, bak, bcl-XL/XS, bcl-2, bcl-w, and bfl-1), and effector family (caspases 1, 2, 3, 6, 7, 8, 11, and 12). cDNA probes for ribosomal protein L32 and glyceraldehyde-3-phosphate dehydrogenase were included as internal controls.

Labeled antisense RNA probes were synthesized from cDNA multiprobe templates with the use of [*-32P]UTP in an in vitro transcription reaction performed according to the manufacturer’s instructions. The labeled RNA probes were hybridized for 16 h at 56°C with samples of 10 μg of total RNA extracted from control, ligated, and contralateral kidneys. The hybridized products were treated with a digestion mixture, including RNase and proteinase. During this procedure, the unhybridized RNA and the free RNA probes were digested but the hybridized RNA were protected from digestion (RNase protection assay). The hybridized RNA were heat-denatured and electrophoresed on polyacrylamide gel for 2 h, 50 W at 45 to 50°C. Gels were dried and exposed to x-ray film at -70°C. The resultant bands were scanned and quantified using PhotoShop and the University of Texas Health Science Center, San Antonio, Image Tool program. Band intensity was normalized to that of L32 in the same reaction.

Statistical Analyses

The morphometric data and the frequency of apoptosis were expressed as mean ± SD. Statistical differences between p53+/+ and p53-deficient mice groups were evaluated with the use of Kruskal-Wallis analysis. *P < 0.05 was considered statistically significant.

Results

p53 Genotypes of Experimental Mice

Southern blot clearly identified three distinct genotypes (p53+/+, p53+/-, and p53−/−). p53+/+ mice had only the wild type p53 gene; p53−/− mice had only the mutant p53 gene; and p53+/- mice had both (Figure 1).

Pathologic Changes of COU

The control and contralateral kidneys from p53+/+, p53+/-, and p53−/− mice were histologically normal (Figure 2A). Regardless of the underlying genotype, obstructed kidneys uniformly developed progressive tubulointerstitial changes characteristic of COU (Figure 2, B through D). The tubular changes included atrophy, dilation, simplification of the tubular epithelium, and tubular cell apoptosis. The interstitial changes included fibroblast proliferation, fibrosis, inflammatory cell infiltration, and apoptosis of interstitial cells. The glomeruli and blood vessels remained intact throughout the experimental period. Although the spectrum of changes was the same for p53+/+, p53+/-, and p53−/− kidneys, the severity of these changes was different among these genotypes. There was no difference between p53+/+ and p53−/− mice in the severity of tubular atrophy (*P = 0.20) and interstitial widening (*P = 0.83; Figure 3). Compared with p53+/+ mice, obstructed kidneys from p53-deficient mice showed significantly less tubular atrophy (*P = 0.05) and less interstitial widening (*P ≤ 0.05) between days 7 and 15, but no significant differences were noted after day 20 (*P ≥ 0.34).

Apoptosis of Tubular and Interstitial Cells

The control and contralateral kidneys showed rare apoptotic cells, which did not differ among genotypes and experimental duration. (Figure 2, E and I). Obstructed kidneys of each genotype showed increased tubular (Figure 2, F through H) and interstitial (Figure 2, J through L) cell apoptosis, but the frequency of apoptotic cells was influenced by the p53 genotype. Kidneys from p53−/− mice, compared with those from p53+/- mice, showed, at various time points, a similar or mildly decreased frequency of apoptotic cells (up to 8 and 9% for tubular and interstitial cell apoptosis, respectively). However, compared with p53+/+ mice, kidneys from both groups showed a significant attenuation of tubular (44% at day 7 [*P = 0.02] and 70% at day 15 [*P = 0.02]) and interstitial cell apoptosis (50% at day 15 [*P = 0.009]). In addition, p53-deficient kidneys showed an earlier peak of tubular cell apoptosis (day 4 for p53−/− and p53+/− versus day 15 for p53+/+) and a later peak of interstitial cell apoptosis (day 20 for p53−/− and p53+/+ versus day 15 for p53+/+; Figure 4).

p53 mRNA Expression and Correlation with Apoptosis

The ligated kidneys of the p53+/+ mice showed a marked increase of p53 mRNA compared with control or unligated contralateral kidneys. This increase was observed day 4, peaked between days 7 and 15, and decreased and returned to the control level at day 30. The pattern of p53 mRNA expression paralleled that of renal cell apoptosis. A slight increase of p53 mRNA was detected in ligated kidneys of p53+/− mice, in a pattern similar to that of p53+/+ mice. p53 mRNA was
not detected in kidneys of p53\(^{1/2}\) mice, irrespective of experimental protocol (Figure 5).

**RNase Protection Assay for Apoptosis-Related Molecules**

**Death Receptor Family.** The Fas ligand mRNA showed a pattern of expression similar for all three genotypes. It was not seen in control or contralateral kidneys but was increased mildly after ureteric ligation. A baseline level of mRNA of other molecules of the death receptor family was noted in control or contralateral kidneys; this level was less in p53\(^{+/−}\) and p53\(^{−/−}\) mice than in p53\(^{+/+}\) mice. Ureteric ligation induced increased expression of these molecules; however, this expression was more pronounced in p53\(^{+/−}\) and p53\(^{−/−}\)”

*Figure 2. Histologic changes. Compared with the control kidney (A), marked tubulointerstitial changes were noted in the ligated kidneys of all genotypes (B through D). Rare apoptotic cells were noted in the control kidney (E and I). Although ligated kidneys from all genotypes showed increased apoptosis of tubular cells (F through H) and interstitial cells (J through L), both p53\(^{+/−}\) and p53\(^{−/−}\) mice showed less apoptosis than did p53\(^{+/+}\) mice.*
Figure 3. Morphometry of tubular atrophy and interstitial widening. (A) Progressive decrease in tubular diameter was noted in ligated kidneys, regardless of genotypes. The severity of tubular atrophy was similar among mice of the different genotypes. Compared with those from p53+/+ mice, the ligated kidneys from p53-deficient mice displayed significantly less tubular atrophy at days 7 and 15: *, P < 0.05. (B) Similar observations were made for interstitial volume of different genotypes. Significantly attenuated interstitial fibrosis was noted in the ligated kidneys from p53-deficient mice at day 15; *, P < 0.05.

Figure 4. Renal cell apoptosis. (A) Ligated kidneys from p53+/− and p53−/− mice shared the same pattern and frequency of tubular cell apoptosis. A significant attenuation of tubular cell apoptosis, reaching 44% at day 7 and 70% at day 15, was noted in both genotypes, compared with p53+/+ mice. The peak of tubular cell apoptosis in p53+/− and p53−/− mice (day 4) was earlier than that of p53+/+ mice (day 15). (B) Interstitial cell apoptosis was attenuated equally in ligated kidneys from p53+/− and p53−/− mice between days 15 and 30, reaching 50% at day 15. The peak of interstitial cell apoptosis in p53+/− and p53−/− mice (day 20) was later than that of p53+/+ mice (day 15). *, P < 0.05.

Figure 5. Northern blot for p53. Compared with the control and contralateral kidneys, the ligated kidneys of p53+/− and p53+/+ mice showed a marked increase of p53 mRNA, which paralleled that of renal cell apoptosis. p53 mRNA was not detected in kidneys from p53−/− mice, regardless of the experimental conditions. CL, contralateral; L, ligated.
mice than in p53+/+ mice. This difference was most evident for TRAIL, TNFR-1, and FAP mRNA (Figure 6).  

Bcl-2 Family. The difference between contralateral or control kidneys on the one hand and ligated kidneys on the other was similar among the three genotypes. Within each genotype, the ligated kidneys, compared with the control or contralateral kidneys, expressed the following pattern of mRNA expression: markedly increased bfl-1 (antiapoptotic molecule), increased bax, bak, and bcl-xS (proapoptotic molecules), decreased bcl-w (antiapoptotic molecule), and unchanged bcl-2, bcl-xL, and bad (Figure 7).  

Caspase Family. The baseline expression of caspases 2, 3, 6, 7, and 8, in control and unobstructed contralateral kidneys, was very low in p53+/− and p53−/− mice but mild in p53+/+ mice. These caspases were increased in ligated kidneys in a pattern similar for all three genotypes. The expression of caspases 1, 11, and 12 at baseline (control and unobstructed contralateral kidneys) was virtually undetectable in all three genotypes. However, these caspases (especially caspase 11) displayed in ligated kidneys distinctive patterns of expression that were significantly different among the various genotypes. Caspase 11 mRNA was markedly increased in ligated kidneys.
from \(p53^{+/-}\) mice and was virtually undetectable in \(p53^{-/-}\) mice at any experimental time point. Caspases 1 and 12 mRNA were increased in the ligated kidneys of all genotypes. However, the increase was much less in \(p53^{+/-}\) and \(p53^{-/-}\) mice than in \(p53^{+/+}\) mice, especially between days 4 and 7.

The time frame of caspase expression in the ligated kidneys was also different among the various genotypes. In \(p53^{+/+}\) mice, they were increased at day 4 after ureteric ligation reached a plateau at day 15, decreased at day 20, and increased again at day 30. However, in \(p53^{+/-}\) and \(p53^{-/-}\) mice, these caspases peaked between days 7 and 15, decreased at day 20, and increased again at day 30 (Figure 8).

**Discussion**

Using mice that were genetically deficient in \(p53\), we demonstrated, for the first time, that renal cell apoptosis in COU is associated with a marked increase of \(p53\) mRNA in the kidney, thus identifying \(p53\) as an important mediator of apoptosis. However, renal cell apoptosis probably proceeds through both \(p53\)-dependent and \(p53\)-independent pathways because apoptosis persisted, albeit at an attenuated level, even when \(p53\) was unequivocally deleted at both DNA and RNA levels. In the setting of \(p53\) gene deficiency, we observed diminished levels of caspases 1, 11, and 12 and a moderate increase in FAP (an apoptosis inhibitor) after ureteric obstruction. Thus, \(p53\) may promote renal cell apoptosis through some novel downstream molecular mechanisms that lead to activation of caspases 1, 11, and 12 and inhibition of FAP. However, ureteric obstruction in the setting of \(p53\) gene deficiency is associated with upregulation of some death receptors (TNFR-1 and TRAIL). Therefore, despite caspase 1, 11, and 12 attenuation, renal cell apoptosis in \(p53\)-deficient mice is probably sustained by high levels of TNFR-1 and TRAIL, in addition to a possible contribution of members of the bcl-2 family. These results suggest that renal cell apoptosis after ureteric obstruction is a complex process, mediated by \(p53\)-dependent as well as \(p53\)-independent pathways. Of note, a full dose of \(p53\) is required for \(p53\)-dependent apoptosis because the degree of mitigation of apoptosis essentially was similar for \(p53^{+/-}\) and \(p53^{-/-}\) genotypes.

\(p53\) protein is a transcription factor that regulates both apoptosis and growth arrest and is one of the best characterized tumor suppressor genes (24,25,28). A marked increase in \(p53\)
expression is observed in cells that are treated with DNA-damaging agents (35,36), in hepatocytes (5), and in neurons (6) that are undergoing apoptosis. Although increased p53 expression has been described briefly in kidneys after ureteric ligation (5,31), our study demonstrates unequivocally a marked increase in p53 mRNA in kidneys of p53\(^{+/+}\) mice after ureteric ligation, in a manner that parallels the incidence of renal cell apoptosis. This observation establishes a clear correlation between the p53 gene and apoptosis in ureteric obstruction. The mechanism by which COU causes increased renal expression of p53 remains unclear, although ischemia, increased renin production, and elevated intratubular pressure may be relevant (31).

p53 can promote apoptosis by a direct effect on apoptosis-related molecules (35–38). Our study showed, for the first time, that kidneys from p53-deficient mice with COU, compared with those from wild-type mice, showed a mild but definite increase (reaching twofold) of FAP, TRAIL, and TNFR-1, whereas other molecules of this family (Fas, Fas ligand, FADD, TRADD, RIP, and FAF) were unchanged. These findings indicate that the apoptotic pathway, mediated by the death receptors, is not only intact but also enhanced in ligated kidneys from p53-deficient mice.

Cell susceptibility to apoptosis is determined partially by the ratio of bcl-2 protein, an apoptosis inhibitor, and bax protein, an apoptosis promoter (15,16,39–41). p53 null mice manifest an attenuated apoptotic response and show a decrease in bax protein with (26) or without (40) an increase of bcl-2 protein.
It is postulated that the protection from apoptosis observed in these mice might be due to a relative predominance of bcl-2 protein (40). These mechanisms, however, do not seem to account for the protection of apoptosis induced by p53 deficiency noted in this study. mRNA levels of bax, bcl-2, and other molecules of the bcl-2 family (bad, bak, bcl-xL/xS, bcl-w, and bfl-1) were not different in the ligated kidneys among the various p53 genotypes.

The most distinctive and novel findings in the current study were a virtual loss of caspase 11 and a marked decrease of caspases 1 and 12 in ligated kidneys of p53-deficient mice. The hierarchy of caspases 1, 11, and 12 within the general apoptotic pathways is not well known, and they are poor substrates for other caspases (22). Murine caspase 11, the murine homolog of human caspase 4, is a member of the interleukin-1β converting enzyme (ICE) subfamily of protease and shares 54% identity with murine ICE/caspase 1 (20). Pro-caspase 11 was found recently to interact physically with pro-ICE, and the expression of caspase 11 is required for activation of ICE (21). Overexpression of caspase 11 induces apoptosis, which could be prevented by bcl-2 and crmA (20). Expression of caspase 11 is highly inducible by lipopolysaccharides (20,21) and cathepsin B (22), suggesting that caspase 11 may have a regulatory role in both apoptosis and inflammatory response (20–22). Findings from the current study confirm the apoptotic roles of caspases 1, 11, and 12, at least for renal cell apoptosis associated with ureter ligation, and suggest, for the first time, that p53 may be essential for their activation. These findings also raise the possibility that p53 might be an upstream regulator of caspase 11 activation and that caspase 11 could be another downstream molecule of the p53-dependent apoptosis pathway. A few reports indicated that caspases are activated by p53 (28,29). Sabbatini et al. (28) demonstrated that all apoptotic pathways downstream of p53-mediated transcription converge upon the activation of caspase 3. Ding et al. (29) reported that p53-dependent activation of caspases in cell-free systems does not require the presence of bax or cytochrome c. However, they did not elucidate the specific relationship between p53 and individual caspases; they just provided indirect evidence for caspase activation, i.e., cleavage of poly (ADP-ribose) polymerase, which is a target for caspase 3 (29). The study presented here, in conjunction with previous reports, suggests that p53 protein can transduce apoptotic signals through protein–protein interactions and that a pathway that is characterized by p53-dependent caspase activation exists (29).

In summary, this study suggests that apoptosis in kidneys with COU is attenuated but not eliminated by deletion of the p53 gene and is both p53 dependent and p53 independent. The p53-dependent pathway may involve p53-mediated transduction of apoptotic signals through the activation of caspases, especially caspases 1, 11, and 12, and this activation requires p53 homozygosity.

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