P53 Mediates the Apoptotic Response to GTP Depletion after Renal Ischemia-Reperfusion: Protective Role of a p53 Inhibitor

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Abstract. Ischemic injury to the kidney is characterized in part by nucleotide depletion and tubular cell death in the form of necrosis or apoptosis. GTP depletion was recently identified as an important inducer of apoptosis during chemical anoxia in vitro and ischemic injury in vivo. It has also been shown that GTP salvage with guanosine prevented apoptosis and protected function. This study investigates the role of p53 in mediating the apoptotic response to GTP depletion. Male Sprague-Dawley rats underwent bilateral renal artery clamp for 30 min followed by reperfusion. p53 protein levels increased significantly in the medulla over 24 h post-ischemia. The provision of guanosine inhibited the increase in p53. Pifithrin-α, a specific inhibitor of p53, mimicked the effects of guanosine. It had no effect on necrosis, yet it prevented apoptosis and protected renal function. Pifithrin-α was protective when given up to 14 h after the ischemic insult. The effects of pifithrin-α on p53 included inhibition of transcriptional activation of downstream p53 targets like p21 and Bax and inhibition of p53 translocation to the mitochondria. Similar results were obtained in cultured renal tubular cells. It is concluded that p53 is an important mediator of apoptosis during states of GTP depletion. Inhibitors of p53 should be considered in the treatment of ischemic renal injury.

Received July 18, 2002. Accepted September 16, 2002.
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DOI: 10.1097/01.ASN.0000040596.23073.01

Journal of the American Society of Nephrology
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Renal Ischemia-Reperfusion (I-R) injury is characterized histologically by inflammation and tubular cell death in the form of necrosis and/or apoptosis (1,2). The relative importance of these two forms of cell death in determining the functional outcome of injury remains poorly understood. Nevertheless, agents that modulate or prevent apoptosis have been shown to be protective after I-R in various tissues and organs. Such agents include antioxidants, α-MSH, caspase inhibitors, and growth factors (3–6). Despite diverse mechanisms of action, all these agents ultimately show potent antia apoptotic properties that account, at least in part, for their protective effects.

We recently identified GTP depletion as an important stimulus for apoptosis after renal I-R in both mice and rats (7,8). We also showed that salvage of GTP levels with guanosine resulted in markedly reduced tubular cell apoptosis and protection from acute renal failure. These studies extended our earlier observations in renal tubular cells in culture that specifically linked the apoptotic phenotype to GTP depletion (9). We now investigate the mechanism by which GTP depletion induces apoptosis.

We hypothesize that the apoptotic response to GTP depletion during I-R is mediated by p53. Indeed, p53 plays a central role as the initiator of the intrinsic apoptotic cascade triggered by a wide variety of insults (10). These include UV irradiation, chemotherapeutic agents, free radicals, hypoxia, and nucleotide depletion (11,12). In addition, a role for p53 in regulating the extrinsic receptor-mediated apoptotic pathway has also been reported (13,14). Thus, p53 is poised as an ideal candidate for mediating apoptosis after I-R, a setting where many of the above insults coexist.

We also investigated the effects of the newly described potent and specific inhibitor of p53, pifithrin-α (PIF). This synthetic compound was recently shown to protect against intestinal epithelial apoptosis after radiation or chemotherapy and was proposed as therapy for the devastating diarrhea that results from these agents (15,16). Our results show that we are able to directly inhibit p53 with PIF after I-R. In fact, PIF had an overall protective profile identical to that we described with guanosine (7). That is, inhibition of p53 protected against apoptosis and resulted in improved renal function without significantly affecting necrosis. Our studies further probe the complex interaction between PIF and p53 by examining the transcriptional activity and cytoplasmic-mitochondrial translocation of p53.

Material and Methods

Renal Ischemia

All animal experimentation was conducted in conformity with the “Guiding Principles for Research Involving Animals and Human Beings.” PIF (2.2 mg/kg; Calbiochem, San Diego, CA) in 0.9% NaCl
or 0.0005% DMSO/0.9% NaCl or an equal volume of 0.9% NaCl or DMSO/0.9% NaCl (placebo) was administered via intraperitoneal injection. The dose of PIF was based on the in vivo and in vitro anti-apoptotic efficacy reported in the literature (16). Male Sprague-Dawley rats weighing 180 to 220 g (Harlan, Indianapolis, IN) were anesthetized with intraperitoneal sodium pentobarbital (50 to 70 mg/kg) and placed on a homeothermic table to maintain core body temperature at 37°C. Both renal pedicles were occluded via a midline incision for 30 or 45 min followed by reperfusion (7,17). Sham surgery consisted of an identical procedure with the exception of application of the microaneurysm clamps. Creatinine was determined by standard picric acid reaction in serum obtained from the tail vein or via cardiac puncture.

**Light Microscopy**

Twenty-four, 48, or 72 h after surgery, kidneys were perfusion-fixed in situ with 4% paraformaldehyde, paraffin-embedded, sectioned at 4 microns, and stained using hematoxylin and eosin.

**Fluorescence Microscopy**

A Zeiss confocal microscope (LSM 510) equipped with UV, argon, and helium lasers was used. Pieces from the in situ fixed kidneys were preserved in 20% sucrose before 10-μm frozen sections were obtained. Some sections were stained for p53 with a primary sheep polyclonal anti-p53 antibody (Ab-7; Oncogene, Boston, MA). This was followed with a secondary unlabeled rabbit anti-sheep IgG (Zymed Lab Inc., San Francisco, CA) and a tertiary Texas Red labeled donkey anti-rabbit IgG (Jackson Lab., West Grove, PA). Mitochondria were stained with anti-cytochrome c oxidase (anti-cox) mouse monoclonal antibody (Molecular Probes, Eugene, OR) and a secondary goat anti-mouse IgG, FITC-conjugated (Jackson Lab.). Finally, nuclei were stained with the nuclear dye To-Pro-3 iodide (Molecular Probes). Images were collected and analyzed with Zeiss LSM software and MetaMorph (Universal Imaging Corporation). In addition, we used Corr3D, a software program developed in our division by Chris Constantine. It allows the quantitation of overlap between pixels from different channels and thus yields an accurate estimate of colocalization (values ranging from 0 [no colocalization] to 1 [100% colocalization]). It can be applied to any field (cytoplasm alone, nucleus alone, etc.) by applying a mask to exclude surrounding areas.

Separate sections were stained with TUNEL reagent (Promega, Madison, WI) and DAPI for in situ apoptosis detection. In brief, 10-μm frozen sections were treated with 20 μg/ml proteinase K and then incubated in a nucleotide mixture containing fluorescein-12-dUTP and TdT (terminal deoxynucleotidyl transferase). Positive controls were pretreated with 1 U/ml Dnase, and negative controls were incubated without TdT. TUNEL-positive nuclei were expressed as a percent of total nuclei (DAPI-positive) per field. Six to eight fields per section and 2 to 3 sections per kidney were examined in each experiment (7).

**Western Blots**

In some experiments, tissue harvested form cortex or medulla was obtained at specified time points before or after I-R. Proteins were extracted with standard techniques and measured by Coomassie blue assay (Pierce Chemical, Rockford, IL). They were then resolved on a 15% Tris-HCl gel by electrophoresis, along with MW markers. An identical amount was loaded in each lane for a given experiment.

After electrophoresis, proteins were transferred to a PVDF filter membrane and probed for p53 (Ab-1, mouse monoclonal), p21 (Ab-5, rabbit polyclonal), or Bax (Ab-1, rabbit polyclonal); all from Oncogene, Boston, MA. Appropriate HRP-conjugated secondary antibodies were used along with ECL labels. Densitometry was performed using Quantity One software from Bio-Rad.

**Studies with LLC-PK1 Cells**

A4.8 clones of LLC-PK1 porcine proximal tubule cells were grown in 5% CO₂ at 37°C in DMEM with 10% FBS (Sigma Chemical, St. Louis, MO) and 5 mM glucose. For chemical anoxia, 0.1 μM antimycin A was used in depleted media (DMEM without amino acids, glucose, or serum). Apoptosis was detected on the basis of nuclear morphology using a Zeiss confocal microscope. Both adherent and floating cells were visualized after staining with Hoechst 33342 and propidium iodide as described previously. Cell viability was based on the criteria of trypan blue exclusion and cell adherence (7.9). Apoptosis was also detected with DNA electrophoresis on a 1.2% agarose gel after phenol-chloroform extraction. Immunofluorescence as well as Western blots for p53, p21, and Bax were performed as described above for renal tissues.

**Results**

**Effects of I-R and Guanosine on Renal Cortical and Medullary p53 Protein**

As shown in Figure 1A, p53, which was not detectable in kidneys from sham animals, increased significantly in the medulla 24 h after I-R. This increase in medullary p53 was reduced sixfold with guanosine, a treatment we have previously shown to be effective to control levels one hour after

![Figure 1. Effects of ischemia-reperfusion (I-R) and guanosine on renal cortical and medullary p53 protein.](image-url)
I-R. Guanosine was also effective in inhibiting the increase in p53 in animals subjected to 45 min renal artery clamp instead of the usual 30 min. These data suggest a causal relationship between GTP depletion after I-R and the increase in medullary p53. Figure 1B shows the time course of p53 increase after I-R. p53 levels were detectable as early as 1.5 h after ischemia and peaked at 24 h. p53 levels tended to normalize back to baseline by 48 h after I-R (data not shown).

**Effects of the p53 Inhibitor PIF on Renal Histology and Morphology after I-R**

Histologic evidence of injury in kidney sections removed 24 h after bilateral renal ischemia was no different in the PIF-treated rats as compared with the saline-treated group. Figure 2 shows representative sections from the outer medulla of sham rats (upper panels) demonstrating normal morphology. Comparable sections from saline-treated (middle panels) or PIF-treated (lower panels) rats 24 h after renal ischemia show marked disruption of normal tubular morphology with debris and casts in most tubules. However, many small, condensed, and fragmented nuclei (arrows) characteristic of apoptosis were present only in the saline-treated group and not in the PIF-treated group. One such nucleus from each middle section (arrowhead) is shown at a higher magnification in the insets. The apoptotic cells were predominantly tubular and many were found shed in the lumen. Finally, to determine whether administration of PIF at the time of ischemia resulted in more rapid recovery of morphologic evidence of injury, H/E sections were also examined 72 h post-ischemia. The extent of tubular debris, casts, and mitoses in sections from the I-R and I-R + PIF groups was comparable (data not shown).

**Effects of the p53 Inhibitor PIF on Apoptosis after Renal I-R**

To quantify the extent of apoptosis after ischemia and reperfusion, the TUNEL reaction was performed. We have previously validated the specificity of the TUNEL assay for apoptosis in this model by light, fluorescence, and electron microscopic criteria (7). Representative sections of outer medulla are shown in Figure 3. Virtually no TUNEL-positive nuclei were seen in kidney sections from sham rats (upper panel). After bilateral renal ischemia and 24 h of reperfusion, large numbers of TUNEL-positive nuclei were seen in the medulla (middle panels). Nuclear morphology was further evaluated by examining the DAPI channel only (without the FITC channel) at higher magnification. Nuclear morphology (insets) of the TUNEL-positive nuclei demonstrated condensed, fragmented nuclei consistent with apoptosis. In contrast, few TUNEL-positive nuclei were seen in the PIF-treated group after I-R (lower panels). The nuclei in these sections were enlarged and showed faint DAPI staining (insets). Quantification of TUNEL-positive cells showed that treatment with PIF at the time of ischemia resulted in a decrease in TUNEL-positive cells in sections of renal medulla from 27.2 ± 4.5% in the I-R group to 3.9 ± 2.1% in the I-R + PIF group (Figure 4A). These results are similar to those observed after the administration of guanosine before renal ischemia (7). To
examine the effect of PIF on apoptosis at later time points, the TUNEL reaction was performed on tissues removed 48 and 72 h after ischemia. Very few TUNEL-positive cells were found in any of these sections (data not shown).

Effects of the p53 Inhibitor PIF on Renal Function after I-R

To assess the functional significance of the differences in TUNEL-positive nuclei seen, serum urea nitrogen and creatinine were measured 24 h after renal ischemia (Figure 4B). Treatment with PIF at the time of renal ischemia resulted in a mean serum creatinine that was lower than that in the groups that received PIF at the time of ischemia (0 h) and 2, 8, and 14 h after renal ischemia. Mean serum creatinine post-ischemia in the I-R group was 0.3 ± 0.1 mg/dl 48 h post-ischemia (versus 1.8 ± 0.3 in the I-R group) and 0.4 ± 0.72 mg/dl 72 h post-ischemia (versus 1.6 ± 0.3) in the I-R group. Thus, the protective effect of PIF was sustained over a period of at least 72 h.

Effect of the p53 Inhibitor PIF on p53 and its Transcriptional Targets p21 and Bax

PIF is known to bind p53 presumably in the cytoplasm and thus inhibit its transcriptional potential. As shown in Figure 5A, PIF did not reduce p53 levels. Rather, it caused an increase, which likely represents mobilization of otherwise poorly extractable p53. PIF did not directly reduce p53 protein levels but rather inhibited its action; we therefore examined its efficacy in downregulating two classical transcriptional targets of p53, namely p21 and Bax, which are involved in cell cycle control and apoptosis, respectively. Figure 5B shows the effects of I-R and PIF on p21. The changes in p21 after I-R were modest (1.2- to 1.5-fold increase) in both the cortex and medulla. PIF did not reduce p21 levels, but rather inhibited its action. This suggests that PIF may act upstream of p21, regulating its transcriptional activity. Figure 5C shows the effects of I-R and PIF on Bax. The changes in Bax after I-R were modest (1.2- to 1.5-fold increase) in both the cortex and medulla. PIF did not reduce Bax levels, but rather inhibited its action. This suggests that PIF may act upstream of Bax, regulating its transcriptional activity.

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p53

mito

nuclei

merge

control  I-R  I-R + PIF
was very effective in reducing p21 protein after I-R to levels lower than baseline in both cortex and medulla. As shown in Figure 5C, there was no change in Bax protein levels in the cortex and a small (but reproducible) 1.4-fold increase in the medulla after I-R. PIF significantly reduced Bax levels in the medulla (but not the cortex) to below baseline levels.

**Effects of I-R and PIF on the Cellular Localization of p53**

Whereas the changes in p53 protein levels after I-R were significant, those of Bax were comparatively modest. This suggested that p53 might be involved in the apoptotic response to I-R via an additional non-transcriptional effect. We therefore examined the cellular localization of p53 with specific emphasis on nuclear, cytoplasmic, and mitochondrial compartments. As shown in Figure 6, there was no detectable p53 in medullary tubular cells from control animals. This was expected from the Western blot results shown in Figure 1. After I-R, there was a remarkable increase in immunoreactive p53 across all medullary tubules examined. That is, p53 was not restricted to any particular tubular segment. Furthermore, the p53 signal was predominantly (but not exclusively) cytoplasmic and co-localized strongly with the mitochondrial signal. Using Corr3D software, the correlation between p53 and mitochondrial signals was 0.8 ± 0.2 and that between p53 and nuclear signals 0.3 ± 0.1. In the presence of PIF, the p53 signals became more finely granular and co-localized less strongly with the mitochondrial signal (Corr3D score decreased to 0.3 ± 0.2). PIF also modestly reduced nuclear p53 colocalization with a decrease in Corr3D score to 0.2 ± 0.1. These results suggest that the increase in p53 after I-R is mostly cytoplasmic and more specifically mitochondrial. PIF proved very potent in inhibiting this mitochondrial localization of p53.

**Studies with LLC-PK Cells**

Because of the architectural complexity of the kidney and the presence of multiple cell types within any given section, we examined the reproducibility of our results in LLC-PK renal proximal tubular cells in culture. We have previously shown that cell death and apoptosis induced after chemical anoxia/recovery is significantly reduced by the provision of guanosine to restore GTP levels (7). We now show that antimycin A treatment followed by recovery upregulated p53 protein levels as early as 4 h after recovery (Figure 7B). In addition, Figure 7A shows that 50 μM guanosine significantly reduced the increase in p53 by 2.5-fold. Of note is the presence of significant amount of p53 in resting control cells unlike renal tissues in which p53 is undetectable under sham conditions. This is a known characteristic of many immortalized cell lines. Figure 7C shows that, like in renal tissues, PIF does not inhibit the increase in p53 after chemical anoxia. Similarly, modest increases in p21 and Bax were noted after chemical anoxia/recovery and these increases were significantly inhibited by PIF (Figure 7D and 7E).

Figure 8 shows that nuclear condensation and fragmentation typical of apoptosis induced by chemical anoxia (panel B) were totally inhibited by PIF (panel C). Indeed, PIF reduced the number of apoptotic cells after chemical anoxia from 30 ± 5 per field to 4 ± 2 per field (ten fields counted per condition with an average of 50 cells per field). PIF alone had no effect on nuclear morphology (panel D). We confirmed these results with DNA electrophoresis, showing typical laddering after chemical anoxia that is inhibited by PIF or guanosine. Overall, PIF increased cell viability at 24 h from 35 ± 6% (chemical anoxia group) to 88 ± 6% (chemical anoxia + PIF group).
These are identical results to those seen with guanosine (7) and similar to the inhibition of apoptosis with PIF in vivo shown in Figure 3.

Finally, we examined the effects of chemical anoxia and PIF on the cellular localization of p53. As shown in Figure 9, p53 (red) in control cells is predominantly nuclear. Chemical anoxia leads to a remarkable translocation of p53 to the cytoplasm, where it strongly colocalizes with mitochondria (green), resulting in a yellow merge (Corr3D correlation 0.9 ± 0.1). Furthermore, when chemical anoxia was performed in the presence of PIF, p53 remained in the cytoplasm, but the colocalization with mitochondria decreased significantly (Corr3D correlation 0.2 ± 0.1) as shown by distinct red and green rather than yellow fluorescence. These results are very similar to the in vivo observations shown in Figure 6.

**Discussion**

There are many mediators of cell death after renal I-R, and they likely interact in a complex array of signaling pathways that result in tubular cell demise. Recently, we identified the depletion of GTP pools as one such mediator and linked it specifically to the apoptotic phenotype (9). We further showed that enhanced GTP recovery had a profound beneficial effect on cell survival and renal function after chemical anoxia and I-R (7). This beneficial effect strictly correlated with inhibition of apoptosis. In this article, we present strong evidence that p53 is the downstream mediator of apoptosis in the setting of GTP depletion and I-R injury in vivo. All the results were further reproduced in LLC-PK renal tubular cells.

Known as the “the guardian of the genome,” p53 is the most frequently mutated tumor suppressor in many forms of neoplasia. This underscores its importance as the master regulator of cell proliferation and cell death. p53 primarily eliminates unwanted or damaged cells and thus insures the overall integrity of the genome. Its regulation is highly complex and involves interactions with mdm2, alterations in p53 protein levels, and also direct phosphorylation or acetylation (11,12). Once activated, p53 induces cell cycle arrest or apoptosis under many conditions where DNA damage poses the risk of malignant transformation.

The role of p53 in mediating cell death during I-R is more controversial. Such a role is generally accepted for neuronal ischemia but not for cardiac I-R injury (18–21). Indeed, recent reports show that apoptosis after cardiac I-R is p53-independent (22,23). Thus, the involvement of p53 could be tissue or organ-specific. The data on p53 after renal I-R is even more scarce. Although some reports propose a potential role for p53, others present data showing that cell death after I-R is p53-
Figure 9. Effects of antimycin/recovery and PIF on the cellular localization of p53 in cultured renal tubular cells. Cultured LLC-PK₁ renal tubular epithelial cells were harvested 24 h after exposure to antimycin A (0.1 µM for 45 min) with or without pifithrin-α (PIF; 10 µM) as detailed in Materials and Methods. Cells were fixed and immunostained with antibodies to p53 (Texas red–label, red) and the mitochondrial marker cytochrome c oxidase (FITC-label, green). Nuclei were stained with To-Pro-3 iodide (blue). Control cells were maintained in standard media.
independent (24–26). Possible reasons for these discrepancies are discussed below.

In this article, we show a significant increase in p53 protein in the medulla after I-R. The medulla is the primary site of tubular cell apoptosis after I-R. The increase in p53 was prevented by guanosine, a treatment we have previously shown to selectively replete GTP stores and inhibit apoptosis (7). Guanosine was capable of inhibiting this increase in p53, even after 45-min renal artery clamp, a procedure that invariably results in extensive damage and acute renal failure. Although these data support a role for p53 in mediating GTP depletion-induced apoptosis, it still could be simply an association rather than a causal relationship. Our studies with PIF provide more direct proof for a causal role of p53 in apoptosis after I-R.

PIF, a synthetic, potent, and highly specific inhibitor of p53, not only inhibited apoptosis but also reproduced our previous findings with guanosine. That is, the inhibition of apoptosis with PIF was accompanied by an impressive functional protection and by the same lack of effect on necrotic death. Taken together, these findings strongly argue for an important role of p53 in mediating apoptotic cell death after I-R and GTP depletion. They further underscore the importance of apoptosis as a determinant of functional outcome after ischemia, independent of necrosis. The exact mechanisms by which inhibition of apoptosis impact renal function post-ischemia are largely unknown. Possible mechanisms include preservation of tubular integrity and thus preservation of tubuloglomerular feedback. Furthermore, shed apoptotic cells could contribute both to tubular obstruction and the formation of gaps in the tubular epithelium resulting in backleak. Recently, a protective effect of PIF was shown after neuronal ischemia (27). To our knowledge, this is the first report showing a beneficial effect of p53 inhibition after renal I-R.

The effects of PIF on p53 activity were complex. First, the downregulation of p21 and Bax was expected, as PIF is thought to bind and mobilize p53 in the cytoplasm and prevent its translocation into the nucleus (16). Although downregulation of the pro-apoptotic Bax fits well with the inhibition of apoptosis, the decrease in p21 was more surprising because this cell cycle regulator generally has anti-apoptotic effects (28). However, the impact of p53 activation on cell fate depends on the balance between the anti-apoptotic p21 and the pro-apoptotic Bax (29). Our data suggest that the Bax effects dominate after renal I-R and an inhibition of p53 and Bax with PIF has an overall beneficial effect despite the reduction in p21. Megyesi et al. (25) have shown that p21 activation is p53-independent. However, their studies were performed in p53 null mice. In these genetically altered mice, other p53-independent pathways that activate p21 could have been induced when p53 is absent. Rapid inhibition of p53 with PIF in wild-type animals might not permit sufficient time for these other pathways to become activated and to stimulate p21.

The other surprising finding in this article is that the increase in p53 protein after I-R is, to a large extent, cytoplasmic. This might explain the lack of increase in p53 reported by Megyesi et al. (25), as they measured only nuclear p53. Our data further show that cytoplasmic p53 colocalizes strongly with mitochondria. This mitochondrial increase in p53 was recently reported in cell culture after hypoxia (10,30). These authors proposed a novel non-transcriptional mechanism by which p53 causes apoptosis. This mechanism is initiated upon direct translocation of p53 to mitochondria, where it localizes predominantly to the membranous compartment. To our knowledge, our data provide the first demonstration of mitochondrial p53 translocation in vivo after renal I-R. Our data also suggest that such a mechanism is important after renal I-R. Indeed, we show that the protective and anti-apoptotic properties of PIF correlate well with its ability to prevent the colocalization of p53 with mitochondria in addition to inhibiting its transcriptional potential.

In summary, the data in this article and our previous report support a model of apoptotic cell death after I-R in which GTP depletion and p53 activation occur sequentially, leading to cell death. We also show that direct inhibition of p53 with PIF, like GTP salvage with guanosine, confers functional protection in addition to inhibition of apoptosis. However, unlike guanosine, the therapeutic window with PIF is much wider (up to 14 h post-ischemia) due to the incremental accumulation of p53. In fact this therapeutic window is wider than that of most other agents proposed to prevent or treat renal I-R. Therefore, agents that modulate the transcriptional activity of p53 or its mitochondrial translocation should be strongly considered for the treatment of ischemic renal injury.

Acknowledgments
This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases grants 1P50 DK61594–01 (PCD) and 1RO1 DK60495–01A1 (PCD) and a grant INGEN from the Lilly Endowment to Indiana University School of Medicine. Dr. Kelly is the recipient of the National Kidney Foundation Clinical Scientist Award. The authors are grateful to Bruce Molitoris for support and advice throughout this project.

References


In “P53 Mediates the Apoptotic Response to GTP Depletion after Renal Ischemia-Reperfusion: Protective Role of a p53 Inhibitor” by Kelly et al., which appeared in the January 2003 issue of JASN, Figures 8 and 9 were erroneously printed in black and white. They are printed below in color.

**Figure 8.** Effect of antimycin/recovery and treatment with the p53 inhibitor PIF or guanosine (G) on apoptosis in cultured renal tubular cells. A representative fluorescent micrograph of LLC-PK₁ cells demonstrating normal nuclear morphology is shown in panel A. Condensed, fragmented nuclei characteristic of apoptosis are seen after antimycin/recovery (0.1 μM antimycin A for 45 min and 24 h recovery; panel B). Apoptosis was not apparent in the cells treated with PIF (10 μM) before antimycin/recovery, as shown by the normal nuclear morphology in panel C. PIF alone did not alter nuclear morphology (panel D). Apoptosis after antimycin/recovery was also evident by characteristic laddering on DNA gel electrophoresis (panel E). Prevention of laddering was seen after antimycin/recovery in the presence of PIF (1 or 10 μM) or guanosine (G; 50 μM; panel E). m, DNA size markers.
Figure 9. Effects of antimycin/recovery and PIF on the cellular localization of p53 in cultured renal tubular cells. Cultured LLC-PK₁ renal tubular epithelial cells were harvested 24 h after exposure to antimycin A (0.1 μM for 45 min) with or without pifithrin-α (PIF; 10 μM) as detailed in Materials and Methods. Cells were fixed and immunostained with antibodies to p53 (Texas red-label, red) and the mitochondrial marker cytochrome c oxidase (FITC-label, green). Nuclei were stained with To-Pro-3 iodide (blue). Control cells were maintained in standard media.