Stathmin-deficient mice develop fibrosis and show delayed recovery from ischemic-reperfusion injury

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Zahedi, Kamyar, Monica P. Revelo, Sharon Barone, Zhaohui Wang, Kathy Tehrani, David P. Citron, John J. Bissler, Hamid Rabb, and Manoocher Soleimani. Stathmin-deficient mice develop fibrosis and show delayed recovery from ischemic-reperfusion injury. Am J Physiol Renal Physiol 290: F1559–F1567, 2006. First published January 24, 2006; doi:10.1152/ajprenal.00424.2005.—In kidneys subjected to ischemic reperfusion injury (IRI) stathmin, a tubulin-binding protein involved in the regulation of mitosis, is expressed in dedifferentiated and proliferating renal tubule cells during the recovery phase. To ascertain the role of stathmin in the recovery from ischemic kidney injury, stathmin-deficient (OP18/H11002) and wild-type (WT) animals were subjected to experimental IRI. At 3, 7, and 14 days after reperfusion serum samples and kidneys were collected for the examination of parameters of renal function, morphology, and recovery. Our studies indicate that on day 14 after reperfusion OP18/H11002 mice have significant renal failure, whereas the creatinine levels of WT animals have returned to baseline. Compared with WT animals OP18/H11002 mice had more extensive tubular fibrosis. The examination of proliferating cell nuclear antigen expression indicated that OP18/H11002 animals have increased proliferative or DNA repair activity for a more prolonged duration. The OP18/H11002 animals also had an increased number of tubules with apoptotic cells. These results suggest that in stathmin-deficient mice subjected to IRI, the aberrant regulation of cell cycle progression, not observed under normal conditions, impairs or at least delays the process of tubular repair and recovery after acute renal injury.

acute renal failure; cell proliferation; renal function; fibrosis

ACUTE TUBULAR necrosis (ATN) resulting from ischemia-reperfusion injury (IRI) is among the major causes of renal failure and morbidity in hospitalized patients (4). Tissue damage in IRI is caused by the disruption of blood flow to the kidney (ischemia) leading to reduced oxygen levels, ATP depletion, and cellular acidosis. Reestablishment of blood flow (reperfusion) further exacerbates the tissue destruction via production of reactive oxygen intermediates (4, 47). In most instances, the renal tubules damage is reversible and the kidneys can recover from ATN (45). The repair phase of IRI is characterized by dedifferentiation of epithelial cells in the denuded tubules, onset of mitosis, relining of the tubules, differentiation of epithelial cells, and recovery of the epithelial (2, 45). The recovery of kidney function subsequent to ATN is dependent on proper progression and regulation of the tubular epithelial cells through the cell cycle; therefore, balancing cell loss and cell proliferation is indispensable to renal repair and the prevention of long-term complications (30, 42).

Stathmin, an 18-kDa phosphoprotein, is expressed in cells that have reentered the cell cycle (1, 3, 5, 8, 40). Stathmin binds to tubulin and regulates microtubule dynamics (33–35). The binding of stathmin to tubulin and its ability to disrupt the microtubules is modulated through its phosphorylation on multiple serine residues (22–26). Inactivation of stathmin is a stepwise process that is primed by phosphorylation of Ser25 and 38 residues of stathmin by p38 and p34(Cdc2) kinases (17). The inactivation of stathmin’s tubulin-binding capacity is then completed via phosphorylation of Ser16 and 63 (17). The Camp-dependent protein kinase and calcium/calmodulin-dependent kinases have been implicated in the mediation of the latter phosphorylation reactions (17, 25, 26, 31). The expression levels and phosphorylation status of stathmin regulate cell division by increasing the instability of interphase microtubules, leading to their depolymerization (32). At the onset of mitosis, stathmin is phosphorylated and its capacity to bind to tubulin and induce microtubular catastrophe is lost. Inactivation of stathmin is among the necessary steps for the polymerization of microtubules and proper formation and functioning of the mitotic spindle (9, 12).

In previous studies, we demonstrated that the expression of stathmin increases in the proximal tubules of kidneys subjected to IRI. Stathmin was expressed by dedifferentiated (vimentin expressing), actively proliferating [proliferating-cell nuclear antigen (PCNA) positive] proximal tubule cells in the corticomedullary junction (46). Based on the location and distribution pattern of stathmin and its coexpression with PCNA and vimentin, which is expressed in the S3 segment in kidney IRI (45), it was concluded that stathmin is also expressed in the S3 segment of the proximal tubule (46). The ablation of stathmin gene in mice does not lead to any phenotypic abnormalities other than development of a mild late onset axonopathy, suggesting that there are redundancies in the system that can compensate for the absence of stathmin (20). However, the consequence of stathmin deficiency in conditions associated with tissue injury and physiological stress has not been examin-
ined. To test this hypothesis, we examined the effect of stathmin deficiency on the outcome of renal IRI.

**MATERIALS AND METHODS**

**IRI.** Animals used for the generation of heterozygote stathmin knockout breeding pairs were the litters of the 14th back cross of heterozygote (OP18+/−) and C57BL/6 wild-type (WT) mice. Mice used for the studies described here were stathmin-deficient (OP18−/−) and WT littermates derived from mating of OP18+/− animals from the aforementioned back cross. Bilateral IRI was induced in WT and OP18−/− animals (28–30 g, n = 6/group) by clamping the renal pedicles with microvascular clamps (45 min) under anesthesia. Completeness of ischemia was verified by blanching of the kidneys, signifying the stoppage of blood flow. The blood flow to the kidneys was reestablished by removal of the clamps (reperfusion) with visual verification of blood return. Animals subjected to sham operation (identical treatment except the renal pedicles were not clamped) were used as controls. During the procedure, animals were well hydrated and their body temperature was controlled at around 94°C using an adjustable heating pad. After ischemia, animals were kept under the veterinarian’s observation. At 3, 7, and 14 days postischemia, animals were killed and blood and kidneys were harvested. Blood samples were centrifuged, and the serum was collected for creatinine measurement. The kidneys were fixed in paraformaldehyde for histopathology and immunohistochemical studies. All studies involving animals were performed according to protocols submitted to and approved by the institutional animal care and use committee at the University of Cincinnati.

**Examination of kidney function.** Serum creatinine levels were measured to determine the integrity of renal function. Blood was collected from animals at the time of death (3, 7, and 14 days). Whole blood was centrifuged at 25°C for 10 min at 10,000 g. Serum was collected and used for creatinine measurement. Creatinine assays were performed on a Roche Cobas Farap automated system (Roche, Nutly, NJ) using the Creatinine 557 kit (Sigma, St. Louis, MO).

**Histological assessment of the kidneys.** At 3, 7, and 14 days, the kidneys were removed and fixed in paraformaldehyde for 24 h. Kidney sections were paraffin embedded, 5-μm sections were cut and stained with hemotxillin and eosin (H&E) or trichrome stains. Tissue sections were examined under low power (×40 magnification) for tubular dilatation, cast formation, calcification, interstitial inflammation, and fibrosis. All these morphological changes were scored semiquantitatively on a 0 to 3 scale (0 = no lesion, 1+ = <25% of parenchyma affected by the lesion, 2+ = >25 to 50% of parenchyma affected by the lesion, 3+ = >50% of parenchyma affected by the lesion). The numerical values from these studies were expressed as means ± SE and analyzed for statistical significance.

**Feulgen staining for determination of cellular DNA content.** For quantitation of nuclear DNA content 5-μm tissue sections were stained using Feulgen stain protocol. Briefly, tissue sections were deproteinized with concentrated HCl followed by staining with Schiff’s reagent (aqueous solution of crystal violet and sulfuric acid). The extent of blue nuclear staining by this staining protocol is directly proportional to the nuclear DNA content.

**Immunohistochemical assessment of the kidney sections.** Paraffin-embedded tissue sections from 3 to 7 animals per time point were examined. Only cells that displayed the characteristic nuclear morphology of apoptosis, including nuclear fragmentation, condensation, and intensely fluorescent nuclei by TUNEL assay, were counted as apoptotic. Cells with TUNEL-positive nuclei, in the absence of the above morphological criteria, were not considered apoptotic. The results indicate the average number of tubules with at least one apoptotic cell per field for five independent fields from at least three different kidneys. The numerical values from these studies are expressed as means ± SE and analyzed for statistical significance.

**Statistical analyses.** Values are expressed as means ± SE. The significance of difference between mean values was examined using ANOVA. A P <0.05 was considered statistically significant.

**RESULTS**

**Expression of stathmin after IRI.** Expression of stathmin increases and reaches its peak levels by 72 h after the induction of IRI (46). Since the studies outlined in this manuscript examine the effect of stathmin deficiency over a period of 14 days after injury, we examined the expression of stathmin on days 3, 7, and 14 after reperfusion. Our results (Fig. 1) indicate that stathmin expression is highly elevated in renal tubules in the corticomedullary region of the kidney on day 3 (Fig. 1b) after IRI. Stathmin expression on days 7 and 14 (Fig. 1, c and d) are below that of day 3, yet are still substantially above that of the sham-operated animals (Fig. 1a).

**Effect of stathmin deficiency on weight, mortality, and renal function of animals subjected to IRI.** All animals were weighed before surgery (day 0) and every day after the surgery. Examination of the weight of the animals indicates that although both groups experience early weight loss after the induction of IRI, the OP18−/− animals show a more sustained but not statistically significant weight loss than the WT animals (data not shown). The mortality rate of the animals subjected to IRI (OP18−/−: 4 dead and WT, 5 dead) was higher than sham-operated animals (OP18−/−: 2 dead and WT, 2 dead). Our data indicate that the mortality rates of the WT and OP18−/− animals during these studies were similar.

The renal function of the WT and OP18−/− animals were examined at 3, 7, and 14 days after IRI by determining the serum creatinine levels (Fig. 2). Our results indicate that the serum creatinine levels increase by day 3 in both OP18−/−...
and WT animals subjected to IRI (0.5 ± 0.04 to 1.3 ± 0.2 in OP18−/− and 0.4 ± 0.1 to 1.7 ± 0.6 mg/dl in WT). On day 7, the serum creatinine remained elevated in both WT (0.9 ± 0.1 mg/dl) and OP18−/− (1.0 ± 0.1 mg/dl) animals. On day 14 OP18−/− animals had significantly elevated levels of serum creatinine (1.1 ± 0.1 mg/dl); in comparison, the serum creatinine levels of WT animals had returned to near normal (0.5 ± 0.03 mg/dl). The comparison of serum creatinine levels of sham operated WT and OP18−/− animals (0.4 ± 0.1 and 0.5 ± 0.04) indicate that in the absence of renal injury the serum creatinine levels of these animals are practically identical. Our results suggest that during the early time points of injury (days 3 and 7) the renal function of WT and OP18−/− animals are similarly affected. However, by day 14 when the renal function of WT animals is back to normal the renal function of OP18−/− mice is worsening and serum creatinine levels (1.1 ± 0.03 mg/dl) are significantly higher than the WT (0.5 ± 0.03 mg/dl) animals (Fig. 2).

**Effect of stathmin deficiency on kidney histopathology after IRI.** The kidneys from WT and OP18−/− mice under normal conditions and after IRI on days 3, 7, and 14 were examined. The kidneys were examined and scored for various injury criteria as outlined in MATERIALS AND METHODS. While the histology of uninjured kidneys is similar in both WT and OP18−/− mice, there are a number of differences in the kidneys of animals subjected to IRI. The kidneys of OP18−/− animals subjected to IRI on day 3 show a higher degree of vacuolization of the tubular epithelium (Fig. 3, D, large arrowheads) than the kidney tubules of the WT animals (Fig. 3, C, large arrowheads). Examination of day 7 kidney sections indicated that both WT and OP18−/− animals had ongoing tissue injury including mild fibrotic changes and some calcification within the tubules; however, the histology of the kidneys were not significantly different from one group to another. On day 14 some OP18−/− animals (2 of 5 animals) exhibited calcification within the kidney tubules (Fig. 3H, diamond marker) and all had significantly increased interstitial fibrosis at the corticomedullary region of the kidney (Fig. 3H, long arrowheads). Calcification and fibrosis were absent in the tubules of the WT animals (Fig. 3). The extent of tissue damage was compared using a scoring matrix described in MATERIALS AND METHODS. Comparison of the scores in Table 1 suggests that the extent of tissue damage on days 3 and 7 were similar in the WT and OP18−/− animals. However, the day 7 samples from OP18−/− and WT animals show slightly increased fibrotic response compared with day 3 and control animals of either genotype. On trichrome stain (Fig. 4), the samples from control kidneys obtained from WT and OP18−/− animals show no increase in fibrous material. On day 14, the kidney from the OP18−/− animals show increased peritubular staining (Fig. 4, blue areas and Table 1) consistent with increased matrix deposition and early stages of fibrous organization. The kidney sections of WT animals on day 14 after IRI (Fig. 4, blue areas and Table 1) were either normal and did not show any fibrotic changes or had low levels of fibrosis comparable to day 7 animals. Comparison of day 14 pathology scores indicate that OP18−/− animals have significant fibrotic response and increased tissue damage while the WT animals seem to have recovered from the injury. These results are in agreement with the renal function data (Fig. 2).

Whereas nuclei were of uniform size in sham-operated WT and OP18−/− animals, examination of H&E-stained sections
of kidneys subjected to IRI revealed a greater preponderance of enlarged nuclei in the kidneys of OP18−/− compared with WT animals. To determine whether these enlarged nuclei represented polyploid cells (cells with increased DNA content), we performed Feulgen staining on kidney sections from sham-operated and day 14 post-IRI OP18−/− and WT animals. One hundred tubules per section were examined for the presence of cells with enlarged or more intensely stained nuclei. Kidneys of sham-operated animals of both genotypes did not have any cells with enlarged or more intensely stained nuclei. Fuelgen staining confirmed the presence of an increased number of cells with enlarged nuclei in the kidneys of OP18−/− and WT animals subjected to renal IRI. Our results indicate that the number of tubules with one or more cells containing enlarged nuclei is significantly higher in OP18−/− compared with WT animals (Fig. 5). Examination of the intensities of nuclear staining, however, was inconclusive and did not point to any differences in the number of polyploid cells in the kidneys of OP18−/− and WT animals after IRI.

Effect of stathmin deficiency on the proliferative and apoptotic response of tubular epithelial cells. Kidneys of WT and OP18−/− sham-operated (control) mice and mice exposed to IRI were examined for a proliferative response (PCNA expression). Our results indicate that compared with OP18−/− mice, WT mice mount a somewhat more vigorous early (day 3) proliferative/DNA repair response (Fig. 6, c and d). Comparison of the proliferative response of the two genotypes on day 7 indicates that they are similar in magnitude (Fig. 6, e and f).
while on day 14 the frequency of PCNA-positive cells (proliferating or cells undergoing DNA repair) are noticeably higher in OP18−/− animals (Fig. 6, g and h). Quantitation of the proliferative response (Table 2), based on the methodology outlined in the Materials and Methods Section, also indicates that animals from both genotypes mount a similar proliferative response early on in IRI (days 3 and 7). While both WT and OP18−/− animals have increased numbers of PCNA tubules as late as day 14 after IRI the latter have a significantly enhanced proliferative response.

Examination of kidney sections for apoptotic cells (Table 3) revealed that the magnitude of apoptotic response on day 3 after IRI is similar in WT and OP18−/− animals. On day 7 the number of tubules with apoptotic cells increases in both WT and OP18−/− animals. Comparison of the apoptotic activity in the two genotypes suggests that the WT animal has marginally higher apoptotic rate than the OP18−/− animals (Table 3). The examination of day 14 samples indicates that the number tubules with one or more TUNEL positive cells are significantly higher in the OP18−/− animals compared with WT animals (Table 3). These results indicate the presence of an imbalance in the proliferative and apoptotic response in OP18−/− animals that may contribute to delayed or lack of recovery from IRI.

**DISCUSSION**

In previous studies, we demonstrated that the expression of stathmin increases during the recovery phase in kidneys subjected to IRI (46). Stathmin is primarily expressed in the proximal tubular epithelium by cells that also express vimentin and PCNA (46). These results indicate that stathmin is a marker of cells that have dedifferentiated, reentered the cell cycle and may be involved in the recovery process. Stathmin is a tubulin-binding phosphoprotein that plays an important role in the regulation of spindle formation and mitosis (14, 15, 18, 19, 32). It needs to be deactivated via phosphorylation of its serine residues in order for the cells to progress through mitosis. Overexpression of WT or non-phosphorylatable stathmin leads to reduced growth rate and in some cases to G2 to M blockage (14, 15, 18, 19). Paradoxically, a reduction in stathmin levels also leads to abnormal formation of the mitotic spindle and accumulation of cells in the G2 to M phase (12, 23, 32). These dichoto-

Table 1. Histological evaluation of kidneys from WT and OP18−/− animals after IR

<table>
<thead>
<tr>
<th></th>
<th>WT Control (n = 3)</th>
<th>OP18−/− Control (n = 3)</th>
<th>WT Day 3 IRI (n = 4)</th>
<th>OP18−/− Day 3 IRI (n = 4)</th>
<th>WT Day 7 IRI (n = 5)</th>
<th>OP18−/− Day 7 IRI (n = 5)</th>
<th>WT Day 14 IRI (n = 14)</th>
<th>OP18−/− Day 14 IRI (n = 5)</th>
</tr>
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<tbody>
<tr>
<td>Cast</td>
<td>0</td>
<td>0</td>
<td>2.5 ± 0.5</td>
<td>2.75 ± 0.25</td>
<td>1.3 ± 0.7</td>
<td>1.6 ± 0.25</td>
<td>2.0 ± 0.25</td>
<td>1 ± 0</td>
</tr>
<tr>
<td>Calcification</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.7 ± 0.7</td>
<td>0.2 ± 0.2</td>
<td>0</td>
<td>0.8 ± 0.5</td>
<td>0</td>
</tr>
<tr>
<td>Interstitial inflammation</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.3 ± 0.3</td>
<td>0.4 ± 0.25</td>
<td>0.6 ± 0.3*</td>
<td>1.4 ± 0.2*</td>
<td></td>
</tr>
<tr>
<td>Fibrosis</td>
<td>0</td>
<td>0</td>
<td>1 ± 0</td>
<td>1.2 ± 0.2</td>
<td>0.6 ± 0.3†</td>
<td>1.6 ± 0.2†</td>
<td>1 ± 0</td>
<td></td>
</tr>
<tr>
<td>Tubular dilatation</td>
<td>0</td>
<td>0</td>
<td>2.5 ± 0.5</td>
<td>1.25 ± 0.25</td>
<td>1.7 ± 0.3</td>
<td>1.6 ± 0.25</td>
<td>0.25 ± 0.25</td>
<td>1 ± 0</td>
</tr>
<tr>
<td>Avg. total score</td>
<td>0.0 ± 0.5</td>
<td>5 ± 1</td>
<td>4 ± 0.4</td>
<td>5 ± 1.5</td>
<td>5 ± 0.7</td>
<td>1.5 ± 0.95†</td>
<td>5.8 ± 0.22†</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE; n denotes the number of animals examined per group. WT, wild-type; IRI, ischemic-reperfusion injury. The extent of kidney damage was determined by examining the criteria listed in the table and assigning a qualitative score to each section. The Avg. total score is the cumulative average score of all examined criteria for each group. *P < 0.05. †P < 0.03.
mous results may be explained by the role of stathmin in the regulation of microtubule dynamics and formation of mitotic spindle (12, 32).

Based on our previous studies (46), evidence showing that stathmin is involved in the regulation of mitosis (14, 15, 18, 19, 32) and the established role of coordinated regulation of cell cycle in recovery from renal injury (10, 36, 37, 42), we hypothesized that the absence of stathmin adversely affects the tubular repair and recovery process subsequent to renal IRI. To test our hypothesis, WT and OP18−/− mice were subjected to kidney IRI. Our results indicate that OP18−/− and WT animals subjected to renal IRI have similar mortality rates. The examinations of functional and morphological changes in the kidneys indicate that: 1) stathmin-deficient animals develop a more prolonged disease compared with WT animals; 2) the proliferative and apoptotic response of OP18−/− animals is prolonged compared with WT animals; and 3) creatinine levels of WT animals after an initial increase on days 3 and 7 return to normal levels by day 14; in contrast, the serum creatinine levels of OP18−/− animals remain elevated throughout the 14 days of these studies (Fig. 2). These results are consistent with a delayed or impaired tubular repair process and the prolongation of renal dysfunction in stathmin-deficient mice during the recovery phase of IRI.

The most salient features of these studies are the histological abnormalities in kidneys of stathmin-deficient mice at 14 days after reperfusion. Our results indicate that while the normal histology of the kidneys has been restored in WT animals by day 14, the kidneys of OP18−/− animals show increased interstitial fibrosis and deposition of calcium in corticomedullary tubules (Figs. 3H and 4), indicative of severe cell injury and consistent with the persistence of damage in kidney tubules. The onset of fibrosis and presence of renal failure at 14 days of reperfusion (Fig. 3) support the conclusion that the ability of stathmin-deficient animals to recover from kidney IRI is impaired. As previously mentioned, stathmin plays an important role in regulation of the G2-to-M transition. Stathmin deficiency may therefore disrupt the proper progression of proliferating cells through the cell cycle during tubular repair. Studies examining the effect of p21, a regulator of the cell cycle, indicate that its deficiency leads to elevated numbers of polyploid cells with enlarged nuclei (29). Our results indicate an increased number of tubular cells with enlarged nuclei but not an elevated number of polyploid cells. Also of interest are

![Graph](image1.png)

Fig. 5. Effect of stathmin deficiency on the prevalence of proximal tubular epithelial cells with enlarged nuclei. Kidney sections obtained from WT and OP18−/− animals were subjected to Feulgen staining, and the percentage of proximal tubules in the corticomedullary region with at least one epithelial cell with enlarged nucleus in 100 tubules per section (3 different animals) were quantitated. The results indicate that the number of tubules containing cells with enlarged nuclei was significantly higher in OP18−/− animals on day 14 post-IRI.

![Images](image2.png)

Fig. 6. Effect of stathmin deficiency on the proliferative response of tubular epithelial cells. The expression of proliferating-cell nuclear antigen (PCNA), a marker of cell proliferation, was examined in 5-μm-thick sections of kidneys obtained from OP18−/− control (a), WT control (b), OP18−/− day 3 IRI (c), WT day 3 IRI (d), OP18−/− day 7 IRI (e), WT day 7 IRI (f), OP18−/− day 14 IRI (g), and WT day 14 IRI animals (h). Photomicrographs (×400 magnification) at top are representative samples of the sections examined for PCNA expression. Bottom: semiquantitative measure of the extent of PCNA expression in the sections examined.
the differences in the patterns of PCNA expression and apoptosis in the WT and OP18−/− animals, where the latter show a more prolonged duration of PCNA expression (Fig. 6) and apoptotic response (Fig. 6). As a cofactor in the polymerase δ complex, PCNA is required not only for the synthesis of the leading strand during replication (S phase) but also plays an important role in nucleotide excision repair (6, 13, 21, 38, 39). The latent increase in PCNA levels may indicate a delayed proliferative response associated with DNA replication and repair in the tubular repair process. Alternatively, based on the effect of stathmin dysregulation on cell cycle progression (14, 15, 18, 19, 32), it could be hypothesized that the absence of stathmin, which may function as an effector protein that is downstream of Cdc2, could lead to the elimination of a G2-to-M transition block (23). The disruption of this regulatory point may contribute to genomic instability by allowing cells with damaged DNA to complete the cell cycle. These damaged cells, which need to undergo DNA repair, may account for the increase in the numbers of enlarged nuclei, and enhanced expression levels of PCNA observed in the kidneys of OP18−/− animals during the later stages of IRI. A preponderance of irreversibly damaged cells may also manifest itself as an increase in the number of apoptotic cells in the injured kidneys of OP18−/− animals. Our results showing that the number of tubules with apoptotic cells is increased in OP18−/− animals on day 14 seem to support this. A concomitant increase in PCNA expression and the number of apoptotic cells in the kidneys of OP18−/− mice could also be suggestive of the presence of an imbalance in the proliferative and apoptotic response that may contribute to delayed or lack of recovery from IRI. It is plausible that impaired tubulogenesis due to the absence of stathmin may result in aberrant recovery and persistence of injury in the renal tubules of OP18−/− mice after IRI.

Renal epithelial cells in regenerating tubules undergo de-differentiation, reenter the cell cycle, reestablish an intact epithelium and redifferentiate during recovery from acute tubular injury (11, 45). Studies indicate that proper progression through the cell cycle is of paramount importance to the recovery process after renal injury (10, 36, 37, 42). This is supported by a number of studies that show enhanced expression of cell cycle regulatory molecules such as p53, p21 and 14–3-3 in the proximal tubule epithelium of kidneys subjected to ischemic or toxic acute renal injury (16, 29, 30). Further evidence for the role of proper cell cycle progression in recovery from renal injury comes from the examination of the effect of deficiency of p21 and 14–3-3, a scaffolding molecule that organizes various signal transducers into signaling modules (44), on the pathology and outcome of IRI. The deficiency of cell cycle regulators such as p21 in renin IRI leads to increased mortality, prolonged renal dysfunction and more severe renal pathology (29, 30). It is possible that the induction of cell cycle regulators in response to acute renal injury is involved in slowing down the proliferative response to allow reversibly damaged cells to undergo repair before completing the cell cycle (29).

Stathmin is involved in the regulation of microtubule dynamics and plays a pivotal role in the regulation of cell division (14, 15, 18, 19, 32). Inactivation of stathmin’s tubulin-binding capacity depends on its serine phosphorylation, and the modulation of stathmin activity is necessary for proper formation and function of the mitotic spindle (17, 25, 26, 31). Serine phosphorylation and inactivation of stathmin are partially dependent on the activity of Cdc2 which in turn is regulated by p21 (27, 28, 43). It is therefore plausible that stathmin functions as an effector protein downstream of Cdc2, and its absence may short-circuit the regulation of the cell cycle. We propose that the delayed recovery and prolongation of renal dysfunction in OP18−/− animals may be due to dysregulation of the G2-to-M transition in cell division, allowing the severely damaged cells to continue to grow. The death of these damaged cells during the tubular repair process may lead to exacerbation of renal injury and development of fibrosis, therefore hampering the recovery process. The functional and histological alterations in the kidneys of OP18−/− animals subjected to IRI support the hypothesis that stathmin deficiency disrupts or delays the process of tubular repair and regeneration subsequent to renal injury. Based on these studies we suggest that an
imbalance in cell proliferation and cell loss contributes to delayed tubular repair, development of fibrotic response and prolonged loss of renal function in stathmin-deficient mice following IRI. It is plausible that downregulation of proteins such as stathmin or p21, which are involved in cell cycle regulation, in kidneys of patients with acute renal failure may result in delayed recovery or prolonged worsening of kidney function and development of chronic renal insufficiency subsequent to acute ischemic insult. Whether the absence of stathmin leads to the development of permanent and chronic renal failure following IRI is not clear. Studies examining the long-term outcome of IRI in stathmin null animals should answer that question.

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