Renal ischemia-reperfusion injury upregulates histone-modifying enzyme systems and alters histone expression at proinflammatory/profibrotic genes

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Submitted 3 February 2009; accepted in final form 25 February 2009

Zager RA, Johnson AC. Renal ischemia-reperfusion injury upregulates histone-modifying enzyme systems and alters histone expression at proinflammatory/profibrotic genes. *Am J Physiol Renal Physiol* 296: F1032–F1041, 2009. First published March 4, 2009; doi:10.1152/ajprenal.00061.2009.—Ischemic renal injury can produce chronic renal inflammation and fibrosis. This study tested whether ischemia-reperfusion (I/R) activates histone-modifying enzyme systems and alters histone expression at selected proinflammatory/profibrotic genes. CD-1 mice were subjected to 30 min of unilateral I/R. Contralateral kidneys served as controls. At 1, 3, or 7 days of reflow, bilateral nephrectomy was performed. Renal cortices were probed for monocyte chemoattractant protein-1 (MCP-1), transforming growth factor-β1 (TGF-β1), and collagen III mRNAs and cytokine levels. RNA polymerase II (Pol II) binding, which initiates transcription, was quantified at exon 1 of the *MCP-1, TGF-β1, collagen III* genes (chromatin immunoprecipitation assay). Two representative gene-activating histone modifications [histone 3 lysine 4 (H3K4) trimethylation (m3) (H3K4m3); histone 2 variant H2A.Z] were sought. Degrees of binding to two relevant histone-modifying enzymes (Set1, BRG1) to target genes were assessed. Renal cortical Set1, BRG1, and H2A.Z mRNAs were measured. Finally, the potential utility of urinary mRNA concentrations as noninvasive markers of these in vivo processes was tested. I/R caused progressive increases in Pol II binding to *MCP-1, TGF-β1*, and *collagen III* genes. Parallel increases in cognate mRNAs also were expressed. Progressive increases in renal cortical Set1, BRG1, H2A.Z mRNAs, and increased Set1/BRG1 binding to target genes occurred. These changes corresponded with: 1) progressive elevations of H3K4m3 and H2A.Z at each test gene; 2) increases in renal cortical TGF-β1/MCP-1 cytokines; and 3) renal collagen deposition (assessed by histomorphology). Postischemic increases in urinary TGF-β1, MCP-1, Set1, and BRG1 mRNAs were also observed. We conclude that: 1) I/R upregulates histone-modifying enzyme systems, 2) histone modifications at proinflammatory/profibrotic genes can result, and 3) urinary mRNA assessments may have utility for noninvasive monitoring of these in vivo events.

H3K4m3; H2A.Z; Set1; BRG1; MCP-1; TGF-β1; collagen III

IN CONTRAST TO MOST CHRONIC renal diseases, which demonstrate near-inexorable progression, acute tubular necrosis has long been considered to be a largely reversible disease (29). This is due to a remarkable capacity for tubular cell regeneration and tissue repair, leading to restitution of nephron function (6, 29). However, in the case of postischemic acute renal failure, recent studies have begun to modify this view. A variety of laboratory studies have demonstrated that in the delayed aftermath of renal ischemia, persistent renal functional defects (e.g., hypertension, urine concentrating defects, proteinuria) can exist (1–3, 27). Furthermore, in the presence of reduced residual nephron mass (e.g., as induced experimentally by unilateral nephrectomy), renal ischemia may evoke progressive glomerular filtration rate reductions (26). Recent studies suggest a potential clinical correlate of these experimental findings (16, 25, 30, 34). For example, Goldberg and Dennen (9) found that 12.5% of long-term survivors of acute kidney injury remain dialysis dependent. Furthermore, 19–32% of such patients manifest chronic renal disease (9). The reason(s) for this failure of renal functional recovery or for disease progression remain unknown. However, one possibility is an ischemia-induced loss of the renal microvasculature, setting the stage for ongoing renal ischemia, secondary inflammation, and advancing interstitial fibrosis (1–3, 14). Alterations in the function or expression of several proinflammatory [e.g., monocyte chemoattractant protein-1 (MCP-1)], profibrotic [e.g., transforming growth factor-β1 (TGF-β1)], and remodeling genes (e.g., osteopontin, TIMP-1) may participate in these processes (2, 5, 7, 19, 21, 28, 32, 37, 41).

Epigenetic alterations are increasingly recognized as critical determinants of gene function (4, 10, 12, 15, 20, 36, 39). Under basal conditions, genomic DNA is tightly packaged into nucleosomes. These functional chromatin units comprise 164 DNA base pairs that are tightly wrapped around two units each of four different histone cores (H2A, H2B, H3, H4). The resulting tight chromatin structure serves as a functional barrier to gene activation, possibly by retarding the binding of transcription factors to specific promoters and by reducing RNA polymerase II (Pol II) access to gene transcription regions (4, 10, 12, 15, 20, 36, 39). A number of histone modifications have the capacity to relax this tight nucleosome structure, thereby increasing transcription factor and Pol II binding to genomic sites, and thus, increasing transcription rates. Such histone modifications include methylation, acetylation, phosphorylation, and sumoylation, and are mediated by specific chromatin-remodeling enzyme systems (36).

In light of the fact that activation of proinflammatory and profibrotic genes appear to occur following ischemic renal injury, we questioned whether histone modifications might exist at relevant target genes. The purpose of this report is to provide specific evidence in support of this hypothesis. Herein we demonstrate that 1) specific and progressive histone-modifying enzyme systems appear to be activated by renal ischemia-reperfusion, 2) specific histone modifications at proinflammatory/profibrotic genes correlate with these changes, 3) there are associated increases in Pol II binding at these target genes, and 4) increases in both cognate mRNAs and cytokine/chemokine protein levels result. Finally, we suggest that measurement of urinary mRNA levels for these relevant cytokines/
chemokines/and histone-modifying enzymes could potentially serve as real time markers for these in vivo events.

**METHODS**

**Ischemic Renal Injury Protocol**

All experiments were conducted with male CD-1 mice (25–30 g body wt; Charles River Laboratories, Wilmington, MA) that were maintained under deep pentobarbital anesthesia (40–50 mg/kg). The surgical protocol was approved by the institution’s Institutional Animal Care and Use Committee. After induction of anesthesia, a midline abdominal incision was performed and the left renal vascular pedicle was identified and occluded with an atrumatic vascular clamp. After completing 30 min of ischemia, the clamp was released and reperfusion was confirmed by the loss of kidney cyanosis. Body temperature was maintained at 37°C with an external heating source. The abdominal incision was then sutured in two layers, and the mice were allowed to recover from anesthesia. Free food and water access were provided. Either 1, 3, or 7 days post-surgery (n = 6–7 per time point), the mice were reanesthetized and both kidneys were resected. The cortices were dissected at 4°C and were then subjected to either total RNA, protein, or chromatin extraction and used for RT-PCR (20), ELISA (R&D Systems, Minneapolis, MN kits) (42–44), or chromatin immunoprecipitation (ChIP) assay, respectively, as previously described (20, 22). The results from the postischemic kidneys were contrasted with those obtained from the contralateral, uninjured (control) kidneys. The contralateral kidneys served as appropriate controls that were confirmed by comparing results from them vs. normal renal cortical tissue samples obtained from sham-operated animals (n = 2–3 at each time point).

**Renal histology.** Six mice were subjected to the above unilateral ischemia protocol. Either 1 wk or 2 wk later, the kidneys were resected, full-length tissue sections were cut and fixed in 10% formalin and embedded in paraffin, and two micron sections were cut and stained with Masson’s trichrome to gauge degrees of collagen deposition (blue staining; Ref. 19). Degrees of interstitial inflammation were qualitatively assessed.

**Urinary Assessments**

mRNAs. The following experiment was conducted to ascertain whether increases in renal cortical BRG1 and Set1 mRNA levels (see RESULTS) corresponded with increased BRG1 and Set1 expression at target genes. To this end, ChIP was employed. After immunoprecipitation with either anti-BRG1 (cat. no. 07-478; Upstate Biotechnologies, Lake Placid, NY) or anti-Set1 (Bethyl Laboratories, Montgomery, TX), the precipitated chromatin was subjected to qPCR to quantify MCP-1, TGF-β1, or collagen III using the primers presented in Table 2.

**HK-2 Cell Culture Experiments**

It has previously been established that proximal tubular cells can respond to injury with an increase in TGF-β1 and MCP-1 mRNAs (e.g., Ref. 19). However, it is not clear whether collagen III gene and histone-modifying enzymes could potentially serve as real time markers for these in vivo events.

Table 1. *Mouse primers used for RT-PCR on mRNA samples*

<table>
<thead>
<tr>
<th>mRNA</th>
<th>Primer Sequences</th>
<th>Product Size</th>
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<tbody>
<tr>
<td>Collagen III</td>
<td>5'-AAA GGT GAA ACT GGT GAA GGT GGC-3'</td>
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</tr>
<tr>
<td>TGF-β1</td>
<td>5'-GCC ATC TTT CAG CCT TGG TTA GGA-3'</td>
<td>279 bp</td>
</tr>
<tr>
<td>MCP-1</td>
<td>5'-AAA GAC AGC CAC TCA GGC GTA CCA-3'</td>
<td>250 bp</td>
</tr>
<tr>
<td>Setd1a</td>
<td>5'-TCA CTA CGT GCT ACT CAT TCA CCA-3'</td>
<td>282 bp</td>
</tr>
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<td>BRG1</td>
<td>5'-AGG CTT CCA CCA CCA CCA CCA CCA CCA-3'</td>
<td>595 bp</td>
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<td>H2A.Z</td>
<td>5'-TCC CCA TCA GGA ATT TGT GGA-3'</td>
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<td>GAPDH</td>
<td>5'-CGG CTA TTT GCA TGT GCA AAG TGG-3'</td>
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Primers were used to quantify mRNAs in renal cortex. TGF, transforming growth factor; MCP, monocyte chemotactic protein; Setd1A, isoform in mouse; BRG, chromatin-modifying enzyme; H2A.Z, histone 2 variant.
activation may also occur. To this end, cultured human kidney HK-2 cells, maintained in keratinocyte serum-free medium (44), were subject to overnight ATP depletion by incubation with 7.5 mM antimycin A (AA) + 20 mM 2-deoxyglucose to inhibit mitochondrial and glycolytic ATP production, respectively (44). Coincubated, carriertreated cells (i.e., not subjected to ATP depletion) served as controls. The following morning, ATP depletion was reversed by washing the cells with fresh media (to remove the AA and deoxyglucose) and allowing them to recover for 4 h. Total RNA was extracted (44) and assayed by RT-PCR for collagen III mRNA using the primers presented in Table 3. Results were expressed as the ratio to the simultaneously obtained GAPDH product. 

Ratios of GAPDH were calculated from at least three experiments. Pol II binding was observed throughout the 7-day assessment. Primers were used for quantitating genomic samples for the 3 test genes. qPCR, quantifying PCR; ChIP, chromatin immunoprecipitation.

Results

MCP-1 Assessments Following Ischemic Renal Injury

Pol II. As shown in Fig. 1, left, there was a progressive increase in Pol II binding at exon 1 of the MCP-1 gene following ischemic renal injury. As early as 24 h postinjury, a doubling of Pol II binding was noted. By 3 and 7 days postischemia, approximately fivefold and tenfold increases in Pol II binding were observed.

mRNA. The time-dependent increases in Pol II binding to the MCP-1 gene were paralleled by increases in renal cortical MCP-1 mRNA levels (Fig. 1, middle). These were apparent by as early as 24 h postischemia (9-fold increase), and by 7 days postischemia 250-fold MCP-1 mRNA increases were observed.

MCP-1 protein. The above noted increases in Pol II binding and MCP-1 mRNA increases were paralleled by progressive increases in MCP-1 protein levels. At the 7-day time point, approximately 15- to 20-fold elevations were observed (Fig. 1, right).

H3K4m3 and H2A.Z levels at MCP-1 exon 1. H3K4m3 levels at the MCP-1 gene rose progressively with time postischemia (~2X increase by 7 days; Fig 2, left). Progressive, and even more dramatic, increases in H2A.Z levels were observed (~5X increase over controls; Fig. 2, right).

TGF-β1 Assessments Following Ischemic Renal Injury

Pol II. As shown in Fig. 3, left, an approximate doubling of Pol II binding was observed at TGF-β1 exon 1 by 1 day postischemia. A modest, but progressive increase in Pol II binding was observed throughout the 7-day assessment.

mRNA. An approximately threefold increase in TGF-β1 mRNA levels was documented by 1-day postischemia (Fig. 3, center). These levels increased over the course of the experiment, reaching values that were ~10-fold higher than those seen in control kidney samples.

TGF-β1 protein. TGF-β1 protein levels paralleled the changes in its mRNA. A statistically significant increase was documented as early as 24 h postischemia (Fig. 3, right). By the 7-day time point, an approximate threefold increase in TGF-β1 levels was observed.

H3K4m3 and H2A.Z levels at TGF-β1 exon 1. Both H3K4m3 (Fig. 4, left) and H2A.Z levels (Fig. 4, right) paralleled the above changes in Pol II binding and TGF-β1 mRNA/protein levels. Both histone marks progressively increased with time postischemia, reaching two- to threefold elevations by the 7-day time point.

Collagen III Assessments Following Ischemic Renal Injury

Pol II. Pol II binding to exon 1 of the collagen III gene rose progressively during the 7-day time course (Fig. 5, left). Although not statistically elevated by 1 day postischemia, approximately twofold and threefold increases were observed at 3 and 7 days postischemia.

mRNA. Like Pol II binding, collagen III mRNA levels rose progressively with time (Fig. 5, middle), reaching values that were 3X, 5X, and 8X those observed in contralateral control kidneys.

Renal histology. Normal renal histology, as revealed with Masson’s trichrome staining, is presented in Fig. 5, right, top. After 1 wk of ischemia, extensive tubular necrosis, tubular regeneration, cast formation, and interstitial mononuclear cell infiltrates were seen. However, no clear evidence of interstitial fibrosis could be documented. However, by 2 wk postischemia, early collagen deposition was observed, as denoted by streaks of blue staining material (Fig. 5, right, bottom). This was in addition to tubular dropout, marked interstitial mononuclear cell infiltrates, and occasional cast formation. These changes were observed throughout the renal cortex and outer medulla. Glomeruli retained a relatively normal appearance at both 1- and 2-wk postischemic damage.

H3K4m3 and H2A.Z levels at collagen III exon 1. As shown in Fig. 6, progressive increases in both H3K4m3 and H2A.Z levels were observed at collagen III exon 1 over the course of the experiments.

Table 3. Primers for RT-PCR of human kidney-derived HK-2 cells

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<td>5'-AGT CAT GCA GTC CGC GTA GAT CAT-3'</td>
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The primers were used for assessing the impact of reversible ATP depletion on HK-2 cells in culture.

Primers for qPCR on ChIP samples

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<th>Product Size</th>
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<td>Collagen III</td>
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<tr>
<td>exon 1</td>
<td>5'-AGG GAC AAC TCA TGC TAC TCT-3'</td>
<td>110 bp</td>
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<td>MCP-1</td>
<td>5'-AGG GAC AAC TCA TGC TAC TCT-3'</td>
<td>190 bp</td>
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Primers were used for quantitating genomic samples for the 3 test genes. qPCR, quantifying PCR; ChIP, chromatin immunoprecipitation.

Table 2. Mouse primers used for qPCR on ChIP samples

<table>
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<tr>
<th>Genes</th>
<th>Primer Sequences</th>
<th>Product Size</th>
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BRG1 mRNA, Set1 mRNA, and H2A.Z mRNA Levels in Renal Cortex

Renal cortical mRNAs for BRG1, Set1, and H2A.Z increased dramatically in response to ischemic injury (Fig. 7). In the case of BRG1 and SET1, their mRNAs progressively rose with time over the 7-day postischemic period (Fig. 7). In the case of H2A.Z mRNA, an approximate doubling was seen at 3 days posts ischemia, but no further increase was seen at the 7-day time point.

**BRG1 and Set1 Levels at the MCP-1, TGF-β1, and Collagen III Genes**

As shown in Fig. 8, the increases in BRG1 and Set1 mRNAs corresponded with increased binding of these two histone-modifying enzymes at exon 1 of each of the three target genes.

**Urinary Assessments**

TGF-β1, MCP-1, and Set1 mRNAs were detected in baseline urine pellets. In each case, a significant increase over these basal values was observed in the urine pellets obtained 18-h postbilateral ischemic renal damage (Fig. 9). The increase in Set1 mRNA was accompanied by an increase in urinary H3K4m3 protein (its molecular product); whereas H3K4m3 could not be detected in baseline urine samples, two low molecular weight bands (~17 kDa) were observed in each of the postischemic urine samples (Fig. 9, right, top; Western blots).

**HK-2 Cell Experiments**

As shown in Fig. 10, HK-2 cells subjected to a 6-h period of ATP depletion plus overnight ATP recovery (antimycin/de-
oxyglucose washout) manifested substantial increases in both MCP-1 and TGF-β1 mRNAs. Thus, this confirmed previous observations that proximal tubules can, indeed, generate these cytokines. This same 6-h period of ATP depletion/repletion protocol failed to significantly increase collagen III mRNA (not shown). However, when the period of ATP depletion was extended overnight and then followed by 4 h of ATP recovery, a marked increase in collagen III mRNA resulted. Thus, this confirmed that proximal tubules can, indeed, generate collagen III message in response to reversible ATP depletion.

DISCUSSION

It has previously been demonstrated that in the aftermath of experimental acute renal injury, a progressive loss of renal function, a result of ongoing inflammation and fibrosis, can result (e.g., 1, 27). Using the glycerol model of ARF, Nath et al. (19) noted that the chemoattractant cytokine MCP-1 and the profibrotic cytokine TGF-β1 help to mediate this proinflammatory/profibrotic state. The present studies extend Nath’s findings to postischemic ARF, given that progressive increases in renal cortical MCP-1 and TGF-β1 mRNAs/proteins were found. The cell type(s) within kidney that were responsible for these changes remain to be tested. However, given that the proximal tubule is the prime site of ischemic renal damage, it seems likely that it was the major source of these MCP-1/TGF-β1 changes. The findings of increased HK-2 cell TGF-β1 and MCP-1 mRNA expression in response to reversible ATP depletion injury (Fig. 10) support this view.

Because increased tissue mRNA levels can reflect either increased transcription or posttranscriptional mRNA stabiliza-

Fig. 3. Pol II binding at the transforming growth factor-β1 (TGF-β1) gene (exon 1), and corresponding TGF-β1 mRNA and protein levels, 1, 3, or 7 days postischemia. Renal ischemia induced a progressive time-dependent increase in Pol II binding at the TGF-β1 gene (exon 1; left) and a corresponding increase in TGF-β1 mRNA (middle). These changes resulted in a progressive increase in TGF-β1 protein levels. Thus, these results mirrored those observed above for MCP-1 (as shown in Fig. 1).

Fig. 4. Assessments of H3K4m3 and histone variant H2A.Z at exon 1 of the TGF-β1 gene. Progressive increases in both histone marks were apparent over the course of the 7-day experiment.
tion (20), it is difficult to ascertain which of these two processes initiated the observed mRNA increases. Assessment of Pol II binding to target genes can help in this regard, given that Pol II binding initiates transcription and serves as a semiquantitative index of this process (13, 15, 17, 33). Therefore, Pol II binding to target genes was assessed. As early as 1 day postischemia, Pol II elevations at the MCP-1 and TGF-β1 genes were observed. The level of binding progressively increased thereafter, paralleling the increases in mRNA and cytokine levels. Thus, the Pol II data imply that the observed mRNA elevations reflected, at least in part, increased transcription rates.

The ultimate mediator of renal fibrosis is collagen deposition. Utilizing a model of recurrent myohemoglobinuric ARF, Nath et al. (19) demonstrated that collagens I, III, and IV each contribute to this process, based on findings of increases in their respective mRNAs and increased collagen deposition (assessed by Masson trichrome staining). In light of these findings, we selected collagen III as a representative gene to gauge collagen formation rates. As shown in Fig. 5, a progressive increase in collagen III mRNA was observed over the course of the experiments. A parallel and progressive increase in Pol II binding at exon 1 of collagen III was also observed, implying increased transcription. These changes appeared to precede overt collagen deposition, given that the latter was noted at 2 wk, but not at 1 wk, postischemia. This time lag between Pol II binding/mRNA formation vs. collagen detection likely reflects a relative lack of sensitivity of histomorphology, compared with RT-PCR and ChIP assessments. The relative contribution of injured proximal tubules vs. fibroblasts to overall collagen deposition remains unknown. However, the observation that HK-2 cells respond to reversible ATP depletion with a tripling of collagen III mRNA formation implies that proximal tubules can indeed participate in this process.

Multiple transcription factors have been identified that regulate MCP-1, TGF-β1, and collagen gene activity in both

Fig. 5. Assessments of collagen expression in the aftermath of ischemic renal injury. A progressive ischemia-induced increase in Pol II binding was observed at exon 1 of the collagen III gene. This was mirrored by increased collagen III mRNA expression. No direct assessment of collagen III protein could be made. As a substitute, collagen deposition was assessed by Masson trichrome staining at 2-wk postschemia (right, bottom) and in control kidney (right, top). Marked (blue) collagen staining was observed in the aftermath of ischemia, confirming that increasing fibrosis corresponded with the preceding collagen III-mRNA and Pol II results.

Fig. 6. Assessments of H3K4m3 and histone variant H2A.Z at exon 1 of the collagen III gene. The results obtained at collagen III recapitulated those observed at MCP-1 and TGF-β1: a time-dependent progressive increase of both H3K4m3 and H2A.Z levels were observed in postschematic kidneys vs. their contralateral nonischemic controls.
health and disease (e.g., 5, 19, 21). The focus of this study was
to test a different but related issue: whether ischemia-reperfu-
sion evokes gene-activating epigenetic alterations at target
genes. To explore this issue, two such alterations were sought
at exon 1 of the MCP-1, TGF-β1, and collagen III genes:
trimethylation of histone 3 lysine 4 (H3K4m3), and upregu-
lation of the normally suppressed histone variant H2A.Z. As
shown in Figs. 2, 4, and 6, progressive increases in both of
these histone marks were observed at each of the three assessed
genes. The functional significance of these histone alterations
remains to be defined. However, that these histone marks are
associated with gene activation in vitro (15) and that their
levels at target genes in postischemic kidneys were paralleled
by cognate mRNA expression are consistent with a functional
role.

H3K4 is a specific target of the methylating enzyme Set1 (8,
31, 40). Given the progressive postischemic H3K4m3 in-
creases at each target gene, we hypothesized that increased
Set1 expression might exist in postischemic kidneys. To ex-
plor e this possibility, renal cortical Set1 mRNA levels were
measured at 1, 3, and 7 days postsurgery, and as shown
in Fig. 7, progressive increases were observed. To explore
potential functional significance, an increase in Set1 protein
binding to each of the three target genes was sought. At the one
assessed time point (7 days postischemia), elevated Set1 pro-
tein levels were confirmed at each of the three target genes.

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Fig. 7. Set1 (a histone 3-lysine 4-methylat-
ing enzyme), BRG1 (the central catalytic
ATPase of the SWI/SNF chromatin remodel-
ing complex), and H2A.Z mRNAs were
assessed in postischemic and control kidneys
at 1, 3, and 7 days postsurgery. The mRNA
for Set1 rose progressively with time in
postischemic kidneys. Similarly, the mRNA
for BRG1 progressively rose postischemia.
H2A.Z mRNA was elevated at each assessed
time point, but progressive increases from 3
to 7 days were not observed.

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Fig. 8. Measurements of BRG1 and Set1 levels at exon
1 of the MCP-1, TGF-β1, and collagen III genes in
7-day postischemic and control kidneys. Significant
increases in both chromatin remodeling enzymes were
observed at each of the 3 assessed genes.
This is consistent with the hypothesis that Set1 helped mediate the corresponding H3K4m3 increases at these sites. To explore whether other histone-modifying enzymes might also be upregulated postischemia, renal cortical BRG1 mRNA levels, and BRG1 protein binding at target exons, were assessed. As hypothesized, increased BRG1 mRNA levels, and increased BRG1 binding at each target gene, were observed. Finally, we tested whether the H2A.Z increases at MCP-1, TGF-β1, and collagen III were associated with increases in renal cortical H2A.Z mRNA, which would be consistent with enhanced H2A.Z protein expression. Indeed, this was the case, since time-dependent H2A.Z mRNA elevations were observed, paralleling the H2AZ protein increases at each gene. Thus, in composite, we believe that the above discussed results are the first to indicate that 1) histone-modifying systems are upregulated in postischemic ARF; 2) these changes correspond with well-defined, gene-activating epigenetic alterations; and 3) corresponding increases in Pol II binding to proinflammatory/profibrinotic genes result. In sum, these processes may well contribute to enhanced MCP-1, TGF-β1, and collagen III transcription, and ultimately, disease progression.

The final goal of this study was to test the hypothesis that the above-described postischemic intrarenal events potentially can be monitored by measuring specific mRNAs and modified histones in urine. Toward proof of concept, urine samples were collected from mice before and 1 day after bilateral renal ischemia and probed for MCP-1, TGF-β1, Set1, and BRG1 mRNAs (urine pellets). Because pellet mRNA values undoubtedly reflect both recovered cell number as well as individual cell mRNA content, each mRNA was factored by simultaneously determined GAPDH content (i.e., correcting for total cell number). As shown in Fig. 9, significant increases in each of these four mRNAs were found in postischemic urine samples, compared with baseline values. In data not presented, H2A.Z mRNA could also be detected.

Fig. 9. TGF-β1 mRNA, MCP-1 mRNA, Set1, and BRG1 mRNAs in urine pellets obtained just prior to and 18 h after the induction of bilateral ischemic renal injury (25 min). TGF-β1, Set1, and BRG1 mRNAs were detectable in baseline urine samples, and each rose dramatically in response to bilateral ischemia. Conversely, MCP-1 mRNA was barely detectable in baseline urine samples but was readily observed postischemia. All values are expressed as a ratio with simultaneously determined urine pellet GAPDH mRNA. To ascertain whether a corresponding increase in the Set1 product H3K4m3 could also be detected, pre- and postischemic urine samples were probed by Western blot analysis. As depicted just above the Set1 mRNA data columns, H3K4m3 was readily detected in postischemic (i) urine, but not in baseline [control (c)] urine samples.

Fig. 10. MCP-1, TGF-β1, and collagen III mRNA responses to reversible ATP depletion in cultured proximal tubule human kidney (HK-2) cells. Reversible ATP depletion × 6 h + overnight recovery increased both MCP-1 and TGF-β1 mRNAs. Collagen III mRNA also rose in response to reversible ATP depletion, but only after an 18-h challenge, followed by 4 h of recovery.
baseline urine samples. However, no postischemic H2A.Z mRNA elevations were observed (in a sense, serving as a negative control). The modified histone H3K4m3 could also be detected in postischemic urine, but not in baseline samples. However, it is unclear whether postischemic H3K4m3 protein appearance reflects increased renal H3K4m3 production, or just a nonspecific increase in nucleosomal shedding from dying epithelial cells. Indeed, this latter possibility suggests that assessments of urinary mRNA, with simultaneous factoring by GAPDH mRNA, will provide for a more specific assessment of real time intrarenal events compared with just urinary histone protein quantitation.

In conclusion, the results of the present study provide the first evidence that renal ischemia-reperfusion evokes progressive increases in histone-modifying enzyme (Set1, BRG1) expression and progressive histone modifications (H3K4m3, H2A.Z) at specific proinflammatory/proangiogenic genes (MCP-1, TGF-β1, collagen III). Corresponding elevations of Pol II recruitment to the transcribed regions of these genes, coupled with parallel increases in their cognate mRNA and cytokine levels, suggest mechanistic relevance to the propagation of postischemic renal damage. The data also suggest that these in vivo processes can potentially be probed by urinary mRNA, and possibly urinary histone, assessments. Further exploration of these latter possibilities would appear to be promising areas for future clinical as well as experimental investigation.

GRANTS

This work was supported by National Institutes of Health Grants R37-38432 and RO-1-DK-68520.

REFERENCES


