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

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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 of 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 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 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/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 postsurgery (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 the results from the contralateral, uninjured (control) kidneys. That the contralateral kidneys served as appropriate controls was 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.

Tissue Analyses

TGF-β1, MCP-1, and collagen III mRNA assessments. The mRNAs for TGF-β1, MCP-1, and collagen III were assessed by ChIP assay, as previously described (20). The mRNAs were factored by simultaneously obtained GAPDH mRNA.

RESULTS

histone-modifying mRNAs. Renal cortical RNA was extracted from kidney samples using RNA Plus-Mini (Qiagen; Valencia, CA). The mRNAs were subjected to competitive RT-PCR for the mRNA of the chromatin-modifying enzyme BRG1 (11, 18, 23, 24, 35, 38, 45), for the histone-methylating enzyme Set1 (Setd1A isoform in mouse), and for H2AZ (20, 38). Therefore, their levels at exon 1 of the TGF-β1, MCP-1, and collagen III genes were assessed by ChIP assay, as previously described (20). H3K4m3 and H2AZ antibodies were obtained from Abcam, Cambridge, MA (cat nos. AB8580-100 and AB4174-100, respectively).

Histone-modifying mRNAs. Renal cortical RNA was extracted from kidney samples using RNA Plus-Mini (Qiagen; Valencia, CA). The mRNAs were analyzed by competitive RT-PCR for the mRNA of the chromatin-modifying enzyme BRG1 (11, 18, 23, 24, 35, 38, 45), for the histone-methylating enzyme Set1 (Setd1A isoform in mouse), and for H2AZ (20, 38). The primer sequences used are presented in Table 1. Results were factored by the simultaneously obtained GAPDH mRNA.

BRG1 and Set1 assays at exon 1 of the TGF-β1, MCP-1, and collagen III genes. The following experiment was undertaken 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.

Urinary Assessments

mRNAs. The following experiment was conducted to ascertain whether the above-noted changes in renal mRNA expression might be reflected by parallel changes in urinary pellet mRNAs. To this end, seven mice were anesthetized and a baseline urine sample was obtained from each mouse by manual compression of the urinary bladder. Then, 25 min of bilateral renal ischemia was induced. After 18–18 h of vascular reflow, the mice were reanesthetized and a postischemic urine sample was obtained. The urine samples were centrifuged, and the pellets underwent RNA extraction. They were then probed for TGF-β1, MCP-1, and Set1 mRNAs by RT-PCR, using the primers presented in Table 1. All values were factored by simultaneously determined GAPDH mRNA levels. Note that these experiments utilized bilateral, rather than unilateral, renal ischemia to avoid assay of urine passed by a normal (nonischemic) kidney.

H3K4m3 levels. A supernatant sample from each of the baseline and postischemic urine samples were probe for H3K4m3 levels to determine whether increased excretion might serve as a marker for ischemic renal damage. Western blot analysis with chemiluminescence detection was conducted with the above-noted anti-H3K4m3 antibody (20).

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

<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 CTT GAC GGC-3′</td>
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</tr>
<tr>
<td>TGF-β1</td>
<td>5′-GCT CTA TTA CAC GGT TGC TTA GAA-3′</td>
<td>279 bp</td>
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<tr>
<td>MCP-1</td>
<td>5′-AAA AGC AGC CAC TCA GCC GGA-3′</td>
<td>250 bp</td>
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<tr>
<td>Setd1a</td>
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<td>BRG1</td>
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<td>595 bp</td>
</tr>
<tr>
<td>H2AZ</td>
<td>5′-CTC GCA TCG CAC GGT GCA GCA-3′</td>
<td>253 bp</td>
</tr>
<tr>
<td>GAPDH</td>
<td>5′-TGG CTA ATG GGA GTG TGC TTA GAA-3′</td>
<td>437 bp</td>
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</table>

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; H2AZ, histone 2 variant.

Collagen III mRNA was measured as being representative of the postinjury renal fibrotic response (19, 21). All three mRNAs were factored by simultaneously assessed GAPDH product.

RNA Pol II binding to TGF-β1, MCP-1, and collagen III gene exons. As a gauge of in vivo gene transcription, Pol II binding to exon 1 of TGF-β1, MCP-1, and collagen III genes was assessed using ChIP assay as previously described (20, 22). In brief, chromatin samples were obtained from fragments of formalin-fixed tissues and immunoprecipitated with anti-Pol II antibody (cat. no. GTX25408; Gene Tex, Irvine TX). The immunoprecipitates were then subjected to real time PCR (qPCR) to quantify exon 1 sequences. The results were expressed per amount of chromatin protein added to the reaction. The employed primers used for the qPCR are presented in Table 2.

Trimethylation of lysine 4 of histone H3 and H2A.Z levels at TGF-β1, MCP-1, and collagen III genes. Trimethylation of histone 3 lysine 4 (H3K4) trimethylation (m3) (H3K4m3) is an epigenetic modification that can induce increased Pol II binding to target genes (8, 31, 40). Increases in the normally repressed histone 2 variant H2A.Z can also correspond with, or promote, gene activation (8, 31, 40). Therefore, their levels at exon 1 of the TGF-β1, MCP-1, and collagen III genes were assessed by ChIP assay, as previously described (20). H3K4m3 and H2A.Z antibodies were obtained from Abcam, Cambridge, MA (cat nos. AB8580-100 and AB4174-100, respectively).
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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<tr>
<td>Collagen III</td>
<td>5'-AGG GAC AAC TGA TGG TGG TAC TCT TCT-3'</td>
<td>141 bp</td>
</tr>
<tr>
<td>exon 1</td>
<td>5'-AGG GTC GGA TCA AGA AGG GTG AGA-3'</td>
<td></td>
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<tr>
<td>MCP-1</td>
<td>5'-GGG AAC AGG TGG ATG ATC-3'</td>
<td>110 bp</td>
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<td>exon 1</td>
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<tr>
<td>TGF-β1</td>
<td>5'-GTT GGC AGA TCC TGC GCA AAC TAA-3'</td>
<td>190 bp</td>
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<tr>
<td>exon 1</td>
<td>5'-ATT AGC AGG CGG TGG ATG CCT TTA-3'</td>
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Primers were used for quantitating genomic samples for the 3 test genes. qPCR, quantifying PCR; ChIP, chromatin immunoprecipitation.

activation may also occur. To this end, cultured human kidney HK-2 cells, maintained in keratinocyte serum-free medium (44), were subjected to overnight ATP depletion by incubation with 7.5 μM antimycin A (AA) + 20 mM 2-deoxyglucose to inhibit mitochondrial and glycolytic ATP production, respectively (44). Coincubated, carrier-treated 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 (n = 3 each for control and experimental groups). The impact of 6 h of ATP depletion with overnight ATP recovery on MCP-1 and TGF-β1 mRNAs was also assessed (n, 3 per group) using the primers presented in Table 3.

Calculations and Statistics

All values are presented as means ± 1 SE. Statistical comparisons between paired (injured and uninjured kidneys) data were made by paired Student’s t-test. Otherwise, unpaired t-testing was used. Significance was judged by a P value of <0.05.

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 (~2× increase by 7 days; Fig. 2, left). Progressive, and even more dramatic, increases in H2A.Z levels were observed (~5× 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 3×, 5×, and 8× 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.
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 postischemia, 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 or 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-
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
health and disease (e.g., 5, 19, 21). The focus of this study was to test a different but related issue: whether ischemia-reperfusion 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 upregulation 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 increases at each target gene, we hypothesized that increased Set1 expression might exist in postischemic kidneys. To explore this possibility, renal cortical Set1 mRNA levels were measured at 1, 3, and 7 days postsurgergy, 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 protein levels were confirmed at each of the three target genes.

Fig. 7. Set1 (a histone 3-lysine 4-methylating enzyme), BRG1 (the central catalytic ATPase of the SWI/SNF chromatin remodeling 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.

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 H2AZ increases at MCP-1, TGF-β1, and collagen III were associated with increases in renal cortical H2AZ mRNA, which would be consistent with enhanced H2AZ protein expression. Indeed, this was the case, since time-dependent H2AZ 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/profibrotic 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, H2AZ mRNA could also be detected in
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 H3K43 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/proliferative 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

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