Endogenous BMP-7 is a critical molecular determinant of the reversibility of obstruction-induced renal injuries

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Submitted 31 January 2011; accepted in final form 25 August 2011

Manson SR, Niederhoff RA, Hruska KA, Austin PF. Endogenous BMP-7 is a critical molecular determinant of the reversibility of obstruction-induced renal injuries. Am J Physiol Renal Physiol 301: F1293–F1302, 2011. First published August 31, 2011; doi:10.1152/ajprenal.00071.2011.—Although obstructive uropathies are frequently correctable through surgery, the potential for permanent renal injury remains even following the successful correction of obstructions. Little is known about the intrinsic mechanisms that determine the reversibility of renal injuries. We and others found that exogenous bone morphogenetic protein 7 (BMP-7) inhibits the pathogenesis of renal injury. Here, we examine the role of endogenous BMP-7 in the outcome of renal recovery following the correction of obstructive uropathies using a reversible murine model of ureteral obstruction. The role of BMP-7 was determined by examining the regulation of BMP-7 during renal recovery and by treating with either BMP-7-neutralizing antibodies or exogenous BMP-7. While BMP-7 is upregulated following the correction of obstructions that lead to reversible renal injury, the upregulation of BMP-7 is diminished following the correction of prolonged obstructions that lead to irreversible renal injury. The activation of the BMP-7 pathway is required for several processes that contribute to renal recovery including the suppression of transforming growth factor-β-dependent profibrotic pathways, the restoration of renal architecture, and the resolution of fibrotic changes in the kidney. Importantly, the therapeutic restoration of BMP-7 enhances renal recovery following the correction of prolonged obstructions that typically lead to irreversible renal injury. Together, these findings show that, while BMP-7 plays a critical role in the repair of obstruction-induced renal injuries, the potential for renal recovery from prolonged obstruction is diminished, in part, due to the dysregulation of BMP-7. Accordingly, renal recovery from obstructive uropathies may be optimized through timely intervention and adjuvant approaches to restore BMP-7 activity.

The clinical management of obstructive uropathies represents a significant challenge to the nephrologist/urologist. Although current surgical approaches are frequently able to correct obstructive uropathies, the potential for permanent renal injury remains even following surgical intervention as evidenced by the fact that obstructive uropathies remain a leading cause of renal injury, chronic renal insufficiency, and renal failure (3, 4, 18). Thus, important goals in the development of improved treatment strategies for obstructive uropathies are to identify the molecular mechanisms that determine the outcome of renal recovery following surgical intervention and to develop adjuvant therapeutic approaches to optimize renal recovery in the patient by targeting those critical molecular mechanisms.

Obstructive uropathies result in the loss of renal structure and function through a well-described series of pathological events characterized by apoptosis, inflammation, and fibrosis (3, 4). Importantly, the kidney has the potential to restore renal structure and function following moderate levels of renal injury (5, 13). The repair of renal injuries is mediated in part by a cycle of dedifferentiation, proliferation, and redifferentiation of intrinsic renal cells that promotes the regeneration of renal architecture (10, 12), the recruitment of extrarenal cells that stimulate repair through paracrine mechanisms (9), and by the activation of proteolytic pathways that resolve fibrotic changes in the kidney (13). Nonetheless, in severe renal injuries, the ability of the kidney to recover from renal injury is frequently diminished (3, 4). Little is known about the repair-promoting mechanisms that are impaired during renal recovery in irreversible renal injuries.

At the molecular level, the activation of the transforming growth factor (TGF)-β pathway plays a central role in the pathogenesis of renal injury by promoting apoptosis, epithelial-mesenchymal transformation, an increase in matrix protein synthesis, and other profibrotic events that lead to the disruption of renal structure and function (2). Accordingly, neutralization of TGF-β inhibits the development of obstruction-induced renal injury (6, 11, 15). The importance of the TGF-β pathway in the pathogenesis of renal injury suggests that the downregulation of the TGF-β pathway is likely an important event during renal recovery from injury, although this possibility has not yet been thoroughly examined.

Another member of the TGF-β protein superfamily, bone morphogenetic protein 7 (BMP-7), has been demonstrated to inhibit TGF-β-dependent biological functions (16). Importantly, treatment with exogenous BMP-7 inhibits the development of obstruction-induced renal injury (8, 17, 19). Interestingly, it has been demonstrated that BMP-7 levels are decreased during the development of renal injury, suggesting that the loss of BMP-7 is a critical molecular event during the pathogenesis of renal injuries (1, 22–24, 26).

Furthermore, our previous studies demonstrated that treatment with exogenous BMP-7 during renal recovery promotes the repair of obstruction-induced renal injuries (14). These findings are supported by the findings of Zeisberg et al. (27, 28) that demonstrated that treatment with exogenous BMP-7 reverses the progression of chronic renal injury. While these studies demonstrate that the renal protective effects of BMP-7 extend beyond simply preventing the development of renal injury, it remains to be determined what role endogenous BMP-7 plays in renal recovery following obstruction-induced injury.

In beginning to determine the role of the intrinsic BMP-7 pathway in renal recovery following injury, several important
Fig. 1. Recovery of the kidney following obstruction-induced renal injury. Mice (n = 3 mice/sample) underwent either sham operation, 2 or 7 days (D) of obstruction, or 2 or 7 days of obstruction followed by reversal, and 10 days of recovery (REC). Kidneys were analyzed by Masson’s trichrome staining (×200; left) and type IV collagen immunofluorescence (×200; right; A), interstitial volume quantification (B), tubular volume quantification (C), and kidney collagen content quantification (D). *P < 0.05; **P < 0.01; n.s. denotes P > 0.05. UUO, unilateral ureteral obstruction.
questions remain to be answered: 1) what is the role of endogenous BMP-7 in renal recovery following injury? 2) is the activity of the intrinsic BMP-7 pathway suppressed in renal injuries that the kidney fails to repair? and 3) is pharmacologic manipulation of the BMP-7 pathway an effective therapeutic approach to reactivate the innate repair mechanisms of the kidney in renal injuries that are typically irreversible? Here, we begin to address these questions to better understand the molecular determinants of the reversibility of obstruction-induced renal injuries.

**MATERIALS AND METHODS**

**Reversible unilateral ureteral obstruction.** A unilateral ureteral obstruction was created in 8- to 10-wk-old C57BL/6J mice by placing a vasculature clamp on the proximal ureter (5). The ureteral obstruction was reversed by subsequent removal of the clamp. Restoration of ureteral patency was verified by injecting methylene blue into the renal pelvis and monitoring its clearance to the bladder. As indicated, ureteral patency was verified by injecting methylene blue into the renal pelvis and monitoring its clearance to the bladder. As indicated, kidney samples underwent either sham operation, 2 or 7 days of obstruction, or 2 or 7 days of obstruction followed by reversal, and 10 days of recovery. BMP-7 protein levels were measured by ELISA. **RT-PCR.** Kidneys were pulverized in liquid nitrogen, homogenized in TRIzol (Invitrogen), and RNA was isolated according to product specifications. RT-PCR was conducted using the Superscript RT-PCR system (Invitrogen) using primers specific for α-smooth muscle actin (5'-CTGAGGTGCTATCTCTC-3', 5'-GCGGCGACCCCTATAATAAA-3'), the α1 chain of type I collagen (5'-ACTGGTACATCAGCCCGAAC-3', 5'-GGTGGAAGGATTTACACGA-3'), or GAPDH (5'-ACTCCACTACGGCAAAATTC-3', 5'-CTTCACAATGCGCAAAGT-3').

**ELISA.** Kidneys were pulverized in liquid nitrogen and homogenized in an extraction buffer containing 20 mM Tris-HCl, pH 7.5, 2 M NaCl, 0.1% Tween, and 1 mM EDTA. Protein levels were determined using a BMP-7 ELISA kit (R&D Systems) according to product specifications.

**Immunoblotting.** Kidneys were pulverized in liquid nitrogen and lysed in 83.3 mM Tris, 150 mM NaCl, 4% SDS, and 100 mM DTT supplemented with Complete Protease Inhibitor cocktail and PhosSTOP phosphatase inhibitor cocktail (Roche). Immunoblotting was conducted using rabbit anti-phosho-SMAD 2/3 (Cell Signaling Technol-
Endogenous BMP-7 and the Reversibility of Renal Injuries

Unilateral ureteral obstruction as a model system to study the reversibility of obstruction-induced renal injuries. To better understand the molecular mechanisms that contribute to renal recovery following injury, we utilized a murine model of obstruction-induced renal injury. In this model, a unilateral ureteral obstruction (UUO) is created in mice by placing a vascular clamp on the proximal ureter (5). UUO results in the development of fibrotic changes to glomeruli and the interstitium (Fig. 1A, Table 1) characterized by interstitial expansion (Fig. 1B, P < 0.05; SHAM vs. 2 days UUO and SHAM vs. 7 days UUO, n = 3), the loss of tubular volume (Fig. 1C, P < 0.05; SHAM vs. 2 days UUO and SHAM vs. 7 days UUO, n = 3), and collagen accumulation (Fig. 1D, P < 0.01; SHAM vs. 2 days UUO and SHAM vs. 7 days UUO, n = 3). Importantly, in our murine UUO model, the obstruction can be reversed by subsequent removal of the clamp in a manner that mimics the surgical correction of obstructive uropathies. In examining renal recovery following the correction of obstruction, we found that mice that undergo 2 days of UUO develop fibrotic changes to the kidney but, following the correction of the obstruction and a recovery period, much of the renal damage subsides over the course of 3 days and the kidney is nearly completely repaired within 10 days (Fig. 1A, Table 1). The repair of the kidney is characterized by a reduction in interstitial volume (Fig. 1B, P < 0.05; 2 days UUO vs. 2 days UUO/10 days REC, n = 3), the restoration of tubular volume (Fig. 1C, P < 0.05; 2 days UUO vs. 2 days UUO/10 days REC, n = 3), and a decrease in collagen content (Fig. 1D, P < 0.01; 2 days UUO vs. 2 days UUO/10 days REC, n = 3). In contrast, we found that mice that undergo 7 days of UUO also develop fibrotic changes to the kidney but, following the correction of the obstruction and a recovery period, much of the renal damage persists following even 10 days of recovery (Fig. 1, A–D, P > 0.05; 7 days UUO vs. 7 days UUO/10 days REC, n = 3). Together, these findings demonstrate that the restoration of renal architecture and the resolution of fibrotic changes in the kidney contribute to the repair of obstruction-induced renal injuries. Conversely, these repair-promoting processes are impaired during renal recovery from prolonged obstructions that lead to irreversible renal injury. Thus, we sought to...

Fig. 3. Endogenous BMP-7 function is required for the suppression of transforming growth factor (TGF)-β-dependent profibrotic pathways during renal recovery. Mice (n = 3 mice/sample) underwent either sham operation, 2 days of obstruction, or 2 days of obstruction followed by reversal, and 3 days of recovery. Mice were treated either with 500 μg/kg mouse IgG (control) or 500 μg/kg BMP-7-neutralizing antibodies daily during recovery, as indicated: A: immunoblot analysis for phospho-Smad 2/3 proteins, phospho-Smad 1/5/8 proteins, and GAPDH (control). B: quantification of phospho-Smad 1/5/8 normalized to GAPDH in 3 independent experiments. C: quantification of phospho-Smad 2/3 normalized to GAPDH in 3 independent experiments. BMP-7 ELISA (D), immunoprecipitation for anti-Smad4 (E), followed by immunoblot analysis for either phospho-Smad 2/3, phospho-Smad 1/5/8, or Smad4 (control). F: quantification of phospho-Smad 1/5/8 normalized to Smad 4 in 3 independent experiments. G: quantification of phospho-Smad 2/3 normalized to Smad 4 in 3 independent experiments. RT-PCR for the TGF-β-dependent target genes type I collagen (H) and α-smooth muscle actin (I). Expression levels were normalized as a ratio to GAPDH expression and then values were compared with those obtained in sham-treated mice. *P < 0.05; **P < 0.01; n.s. denotes P > 0.05.
identify the critical molecular mechanisms that determine the reversibility of obstruction-induced renal injuries.

**BMP-7 pathway is activated during renal recovery from renal injuries that are repaired by the kidney but suppressed in irreversible renal injuries.** Based on the previously described findings (14, 27, 28), we hypothesized that the activity of the intrinsic BMP-7 pathway plays an important role in determining the outcome of renal recovery following obstruction. Accordingly, we examined the regulation of endogenous BMP-7. While BMP-7 levels are decreased during the development of renal injury following UUO, we found that BMP-7 levels are upregulated during renal recovery from UUO (Fig. 2, *P* < 0.01; 2 days UUO vs. 2 days UUO/10 days REC, *n* = 3). The upregulation of BMP-7 is observed within 3 days following the correction of obstruction, the time period where the majority of repair also occurs (14). In contrast, BMP-7 levels remain suppressed during renal recovery following prolonged obstruction, even at 10 days following the correction of obstruction (Fig. 2, *P* > 0.05; 7 days UUO vs. 7 days UUO/10 days REC, *n* = 3). Together, these findings are consistent with the possibility that the intrinsic BMP-7 pathway plays an important role in determining the reversibility of obstruction-induced renal injuries.

**Endogenous BMP-7 function is required for the suppression of TGF-β-dependent profibrotic pathways during renal recovery.** To further examine the role of the intrinsic BMP-7 pathway in determining the reversibility of obstruction-induced renal injuries, we next determined the molecular conse-
Fig. 4. Endogenous BMP-7 function is required for the innate ability of the kidney to repair obstruction-induced renal injuries. Mice (n = 3 mice/sample) underwent either sham operation, 2 days of obstruction, or 2 days of obstruction followed by reversal, and 3 days of recovery. Mice were treated either with 500 μg/kg mouse IgG (control) or 500 μg/kg BMP-7-neutralizing antibodies daily during recovery, as indicated. Kidneys were analyzed by Masson’s trichrome staining (×200; left) and type IV collagen immunofluorescence (×200; right; A), interstitial volume quantification (B), tubular volume quantification (C), and kidney collagen content quantification (D). **P < 0.01; ***P < 0.001.
quences of blocking endogenous BMP-7 function during the ongoing repair of renal injuries through treatment with BMP-7-neutralizing antibodies (25). We and others previously showed that BMP-7 inhibits TGF-β-dependent pathways that play a central role in the pathogenesis of renal injury (14, 27). The inhibitory effects of BMP-7 are mediated, at least in part, by the activation of BMP-7-dependent Smad proteins (Smad1/5/8) that compete with TGF-β-dependent Smad proteins (Smad2/3) for binding to the Smad4 protein during the formation of active transcription factor complexes (14). Therefore, we explored the possibility that the activation of the BMP-7 pathway following the correction of obstruction may contribute to the suppression of TGF-β-dependent profibrotic pathways during renal recovery.

While renal recovery from reversible renal injuries is associated with the upregulation of BMP-7 and the activation of the Smad1/5/8 proteins, blocking BMP-7 function by daily injection of 500 μg/kg of BMP-7-neutralizing antibodies prevents the activation of the Smad1/5/8 proteins during renal recovery (Fig. 3, A and B, P < 0.01; 2 days UUO/3 days REC vs. 2 days UUO/3 days REC + BMP-7 bAB, n = 3) despite the continued upregulation of BMP-7 (Fig. 3D, P > 0.05; 2 days UUO/3 days REC vs. 2 days UUO/3 days REC + BMP-7 bAB, n = 3). Importantly, neutralizing BMP-7 function allows the persistence of elevated levels of Smad2/3–Smad4 transcription complexes (Fig. 3, E and G, P < 0.01; 2 days UUO/3 days REC vs. 2 days UUO/3 days REC + BMP-7 bAB, n = 3) and their downstream TGF-β-dependent profibrotic gene products (7, 20) (Fig. 3, H and I, P < 0.05; 2 days UUO/3 days REC vs. 2 days UUO/3 days REC + BMP-7 bAB, n = 3) during renal recovery. Together, these findings demonstrate that the activation of the intrinsic BMP-7 pathway is required for the suppression of TGF-β-dependent profibrotic pathways that occur during renal recovery.

Endogenous BMP-7 function is required for the repair of obstruction-induced renal injuries. To further examine the role of the intrinsic BMP-7 pathway in renal recovery, we next examined the requirement for endogenous BMP-7 function during the ongoing repair of renal injuries by treating mice with BMP-7-neutralizing antibodies during the 3-day time period following the correction of obstruction where the majority of repair occurs (14). Subsequently, we found that treatment with BMP-7-neutralizing antibodies markedly inhibited the repair of obstruction-induced renal injuries (Fig. 4A, Table 2). Treatment with BMP-7-neutralizing antibodies inhibits the restoration of renal architecture as measured by interstitial volume (10.3% relative reduction in interstitial volume compared with a 39.7% relative reduction in untreated mice; Fig. 4B, P < 0.01; 2 days UUO/3 days REC vs. 2 days UUO/3 days REC + BMP-7 bAB, n = 3) and tubular volume (2.9% absolute increase in tubular volume compared with a 24.1% absolute increase in untreated mice; Fig. 4C, P < 0.001; 2 days UUO/3 days REC vs. 2 days UUO/3 days REC + BMP-7 bAB, n = 3). Similarly, while mice that undergo 2 days of UUO followed by correction of the obstruction and a 3-day recovery period exhibit a 32.3% relative reduction in renal collagen content during the recovery period, mice that are treated with BMP-7-neutralizing antibodies during the recovery period exhibit an 11.3% relative reduction in renal collagen content (Fig. 4D, P < 0.01; 2 days UUO/3 days REC vs. 2 days UUO/3 days REC + BMP-7 bAB, n = 3). Together, these findings demonstrate that the activation of the intrinsic BMP-7 pathway is also required for the restoration of renal architecture and the resolution of fibrotic changes in the kidney following the correction of obstruction. Thus, the activation of the intrinsic BMP-7 pathway during renal recovery is required for the innate ability of the kidney to repair renal injuries.

Therapeutic reactivation of the BMP-7 pathway enhances renal recovery from irreversible renal injuries. Given that the intrinsic BMP-7 pathway plays an important role in the repair of renal injuries and that the activity of the intrinsic BMP-7 pathway is suppressed during renal recovery from irreversible renal injuries, we next sought to evaluate the therapeutic efficacy of stimulating the BMP-7 pathway in improving the outcome of renal recovery following irreversible renal injury. Importantly, we found that the restoration of BMP-7 activity through treatment with exogenous BMP-7 stimulated renal recovery following prolonged obstruction (Fig. 5A, Table 3). While mice that undergo 7 days of UUO exhibit little restoration of renal architecture or resolution of fibrotic changes in the kidney during renal recovery, treatment with exogenous BMP-7 following the correction of obstruction moderately enhances the restoration of renal architecture [Fig. 5, B and C, P > 0.05; 7 days UUO/10 days REC vs. 7 days UUO/10 days REC + BMP-7 (REC), n = 3] and the resolution of fibrotic changes in the kidney [Fig. 5D, P < 0.05; 7 days UUO/10 days REC vs. 7 days UUO/10 days REC + BMP-7 (REC), n = 3]. Notably, when treatment with exogenous BMP-7 is administered both during obstruction and following the correction of obstruction, the restoration of renal architecture [Fig. 5, B–C, P < 0.01; 7 days UUO/10 days REC vs. 7 days UUO/10 days REC + BMP-7 (UUO/REC), n = 3] and the resolution of fibrotic changes in the kidney [Fig. 5D, P < 0.001; 7 days UUO/10 days REC vs. 7 days UUO/10 days REC + BMP-7 (UUO/REC), n = 3] are significantly enhanced during renal recovery. Together, these findings demonstrate that the therapeu
tical reactivation of the intrinsic BMP-7 pathway enhances renal recovery from irreversible renal injuries.

### Table 2. Endogenous BMP-7 function is required for the innate ability of the kidney to repair obstruction-induced renal injuries

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Tubular Volume, %</th>
<th>Collagen Content, μg/mg protein</th>
<th>Interstitial Cells, cells/field</th>
<th>Composite Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHAM</td>
<td>77.3 ± 1.8 (0.67 ± 0.58)</td>
<td>0.51 ± 0.19 (0.00 ± 0.00)</td>
<td>20.3 ± 4.2 (0.33 ± 0.58)</td>
<td>0.33 ± 0.39</td>
</tr>
<tr>
<td>2 days UUO</td>
<td>30.4 ± 5.7 (3.00 ± 0.00)</td>
<td>1.68 ± 0.14 (3.00 ± 0.00)</td>
<td>45.0 ± 9.6 (2.00 ± 1.00)</td>
<td>2.67 ± 0.33</td>
</tr>
<tr>
<td>2 days UUO/3 days REC</td>
<td>52.7 ± 5.1 (1.33 ± 0.58)</td>
<td>1.11 ± 0.13 (1.67 ± 0.58)</td>
<td>35.7 ± 7.6 (1.67 ± 0.58)</td>
<td>1.56 ± 0.58</td>
</tr>
<tr>
<td>2 days UUO/3 days REC + BMP-7 bAB</td>
<td>32.3 ± 2.9 (3.00 ± 0.00)</td>
<td>1.54 ± 0.09 (3.00 ± 0.00)</td>
<td>42.0 ± 5.6 (2.00 ± 0.00)</td>
<td>2.67 ± 0.00 *P &lt; 0.01</td>
</tr>
</tbody>
</table>

Values are means ± SE. Samples from Fig. 4 were assigned a numerical score ranging from 0 to 3 (0 - normal, 1 - mild, 2 - moderate, 3 - severe) related to changes in tubular/interstitial volume, collagen content, and number of interstitial cells. Composite scores represent the average of the individual scores for each sample. Statistical significance is indicated for the (*) 2 days UUO/3 days REC; 2 days UUO/3 days REC + bone morphogenetic protein 7 (BMP-7) bAB sample set.
Therapeutic reactivation of the BMP-7 pathway enhances renal recovery following the correction of prolonged obstructions that typically result in irreversible renal injury

<table>
<thead>
<tr>
<th>Tubular Volume, %</th>
<th>Collagen Content, μg/mg protein</th>
<th>Interstitial Cells, cells/field</th>
<th>Composite Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHAM</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>7 days UUO</td>
<td>79.7 ± 3.2 (0.33 ± 0.58)</td>
<td>0.64 ± 0.12 (0.17 ± 0.41)</td>
<td>16.0 ± 3.6 (0.00 ± 0.00)</td>
</tr>
<tr>
<td>7 days UUO/10 days REC</td>
<td>25.7 ± 8.0 (3.00 ± 0.00)</td>
<td>1.62 ± 0.24 (2.83 ± 0.41)</td>
<td>52.0 ± 9.5 (2.67 ± 0.58)</td>
</tr>
<tr>
<td>7 days UUO/10 days REC + BMP-7 (REC)</td>
<td>34.3 ± 4.5 (3.00 ± 0.00)</td>
<td>1.42 ± 0.21 (2.83 ± 0.41)</td>
<td>60.7 ± 7.8 (3.00 ± 0.00)</td>
</tr>
<tr>
<td>7 days UUO/10 days REC + BMP-7 (REC)</td>
<td>47.7 ± 8.1 (3.33 ± 0.58)</td>
<td>1.10 ± 0.17 (1.83 ± 0.75)</td>
<td>57.7 ± 6.0 (3.00 ± 0.00)</td>
</tr>
<tr>
<td>7 days UUO/10 days REC + BMP-7 (UUO/REC)</td>
<td>58.3 ± 6.4 (3.33 ± 0.58)</td>
<td>0.82 ± 0.15 (0.67 ± 0.82)</td>
<td>57.3 ± 8.1 (2.67 ± 0.58)</td>
</tr>
</tbody>
</table>

Values are means ± SE. Samples from Fig. 5 were assigned a numerical score ranging from 0 to 3 (0 - normal, 1 - mild, 2 - moderate, 3 - severe) related to changes in tubular/interstitial volume, collagen content, and number of interstitial cells. Composite scores represent the average of the individual scores for each sample. Statistical significance is indicated for the (*) 7 days UUO/10 days REC; 7 days UUO/10 days REC + BMP-7 (REC) and (†) 7 days UUO/10 days REC; 7 days UUO/10 days REC + BMP-7 (UUO/REC) sample sets, respectively.

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**DISCUSSION**

A critical goal in the development of improved treatment strategies for obstructive uropathies is to identify the molecular mechanisms that contribute to renal recovery following the correction of obstructive uropathies. Although our study is limited in sample size, we found that BMP-7 function is required for the restoration of renal architecture and the resolution of fibrotic changes in the kidney that occur during renal recovery. Importantly, while the BMP-7 pathway is activated during the repair of obstruction-induced renal injuries, the BMP-7 pathway is suppressed following the correction of prolonged obstructions that result in irreversible renal injury. This suggests that the potential for renal recovery from prolonged obstructions is diminished, in part, due to the dysregulation of the BMP-7 pathway. Indeed, the therapeutic restoration of BMP-7 enhances renal recovery from obstruction-induced renal injuries that are typically irreversible.

While exogenous BMP-7 has been well-established to inhibit the pathogenesis of obstruction-induced renal injury (8, 17, 19), the role of endogenous BMP-7 has remained unclear. Our finding that treatment with BMP-7-neutralizing antibodies impedes the repair of obstruction-induced renal injuries demonstrates that BMP signaling plays an important role in renal recovery following the correction of obstructions. While we cannot rule out the possibility that BMP-7-neutralizing antibodies may affect other BMP family members, the findings that BMP-7 expression is suppressed following irreversible injury and that restoring BMP-7 activity through treatment with exogenous BMP-7 enhances renal recovery strongly suggest that BMP-7 is a critical mediator in kidney repair. Given that the loss of BMP-7 is observed in response to obstructive uropathy, ischemia, chronic inflammation, diabetic nephropathy, and other conditions that lead to renal injury (1, 22–24, 26), the dysregulation of the repair-promoting effects of BMP-7 may contribute to the pathogenesis of a variety of disorders that affect the kidneys and urinary tract.

In the context of the treatment of obstructive uropathies, our findings suggest that timely surgical intervention is necessary, not only to limit the adverse consequences of obstructive uropathies, but also to prevent the erosion of BMP-7-dependent repair mechanisms in the kidney. Our findings show that one important function of the increase in BMP signaling during renal recovery is to activate BMP-7-dependent Smad proteins that competitively inhibit the ability of TGF-β-dependent Smad proteins to bind to Smad 4 and activate the transcription of profibrotic genes that lead to disease progression. Given the conserved nature of TGF-β activation in the response to renal injury, it is likely that TGF-β plays a physiologic role in the early stages of repair that respond to mechanical injury during obstruction, but that the activation of the BMP-7 pathway following the correction of obstruction serves to suppress the TGF-β response, prevent disease progression, and allow the remodeling of the kidney. While the development of irreversible renal injury is associated with the suppression of the BMP-7 pathway, we found that the therapeutic restoration of BMP-7 may serve as an effective adjuvant approach to optimize the innate repair mechanisms of the kidney. Still, given that the effectiveness of treatment with exogenous BMP-7 declines over the course of the progression of renal injury, there is a “therapeutic window” for the utility of BMP-7-targeted approaches to stimulate renal recovery following obstruction-induced injury.

Not only do studies of BMP-7 have potential therapeutic applications, but also studies of BMP-7 may have applications to the diagnosis and evaluation of renal injuries. A critical decision point in the management of obstructive uropathies is the determination of the necessity for intervention. Accordingly, the identification of diagnostic biomarkers is of great utility (3). While many putative biomarkers are indirect measures of renal injury, the development of biomarkers that measure BMP-7 function may provide a direct assessment of the pathways that contribute to renal recovery.

**Table 3. Therapeutic reactivation of the BMP-7 pathway enhances renal recovery following the correction of prolonged obstructions that typically result in irreversible renal injury**

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**Fig. 5. Therapeutic reactivation of the BMP-7 pathway enhances renal recovery following the correction of prolonged obstructions that typically result in irreversible renal injury.** Mice (n = 3 mice/sample) underwent either sham operation, 7 days of obstruction, or 7 days of obstruction followed by reversal, and 10 days of recovery. Mice were treated either with PBS (control) or 300 μg/kg BMP-7 daily during recovery or obstruction and recovery, as indicated. Kidneys were analyzed by Masson’s trichrome staining (×200; left) and type IV collagen immunofluorescence (×200; right; A), interstitial volume quantification (B), tubular volume quantification (C), and kidney collagen content quantification (D). *P < 0.05; **P < 0.01; ***P < 0.001; n.s. denotes P > 0.05.
As we work toward realizing the full potential of the utility of the BMP-7 pathway in the evaluation and treatment of obstructive uropathies and other conditions that lead to renal injury, an important future direction will be to elucidate the molecular mechanisms that regulate BMP-7 levels/activity during the pathogenesis of renal injury. These and other studies aimed toward a better understanding of renal recovery following injury may allow the innate repair mechanisms of the kidney to be evaluated during the diagnosis of renal injuries and manipulated during the treatment of renal injuries.

GRANTS
This research was supported through funding from the Midwest Stone Institute provided to P. F. Austin.

DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

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