# HDAC Dependent Transcriptional Repression of *Bmp-7* Potentiates TGF-β Mediated Renal Fibrosis in Obstructive Uropathy

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**Abbreviations and Acronyms**

α-SMA = α-smooth muscle actin
BMP-7 = bone morphogenic protein 7
COLIα1 = type I collagen, α1 chain
ELISA = enzyme-linked immunosorbent assay
GAPDH = glyceraldehyde-3-phosphate dehydrogenase
HDAC = histone deacetylase
IMCD = inner medullary collecting duct
PCR = polymerase chain reaction
RT-PCR = reverse transcriptase-PCR
SMAD = SMA- and MAD-related protein
TGF-β = transforming growth factor-β
TSA = trichostatin A
UUO = unilateral ureteral obstruction

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**Purpose:** Recombinant BMP-7 inhibits the pathogenesis of renal injury in response to various stimuli. However, little is known about the molecular regulation of endogenous BMP-7 and its renal protective functions. We examined transcriptional regulation of *Bmp-7* and its role in the pathogenesis of renal injury resulting from urinary tract dysfunction.

**Materials and Methods:** Obstruction induced renal injury was modeled in vivo in mice by unilateral ureteral obstruction and in vitro in primary kidney cells by treatment with transforming growth factor-β, a profibrotic cytokine that is increased in the obstructed kidney.

**Results:** Unilateral ureteral obstruction resulted in the loss of BMP-7 expression in conjunction with histone deacetylation and transcriptional repression of the *Bmp-7* promoter. The histone deacetylase inhibitor trichostatin A stimulated *Bmp-7* expression in primary kidney cells. Trichostatin A also inhibited the expression of transforming growth factor-β dependent profibrotic genes in a manner that depended on BMP receptor signaling. These findings extended to the obstructed kidney in vivo, in which trichostatin A treatment restored the expression of *Bmp-7* along with BMP-7 mediated suppression of transforming growth factor-β dependent signaling pathways. Finally, trichostatin A stimulated activation of the BMP-7 pathway the ameliorated obstruction induced renal injury by preventing disruption of the renal architecture and the development of renal fibrosis.

**Conclusions:** These findings show that histone deacetylase dependent repression of *Bmp-7* transcription is a critical event during the pathogenesis of renal injury in obstructive uropathy. Accordingly, treatment with histone deacetylase inhibitors represents a potentially effective strategy to restore BMP-7 expression and its renal protective functions during treatment of obstructive uropathy.

**Key Words:** kidney, ureteral obstruction, bone morphogenetic protein 7, fibrosis, histone deacetylase inhibitors

Obstructive uropathy is a leading cause of pediatric kidney disease, accounting for 16.5% of kidney transplants in children.[1] While the kidney has innate repair mechanisms that can restore renal structure and function after acute injury due to urinary obstruction and other stimuli,[2–4] the clinical manifestation of chronic renal injury stems in part


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from a dysregulated wound healing response, which results in renal fibrosis.[3,4] At the molecular level activation of the TGF-β pathway is classically described as a critical pathological step in the development of renal fibrosis that promotes apoptosis, cellular dedifferentiation, myofibroblast activation and matrix protein synthesis.[5] Given that these processes are involved in injury repair,[4] it is likely that TGF-β also has a physiological role in the early stages of injury repair but when TGF-β activity is dysregulated, it instead promotes disease progression. Nonetheless, mechanisms that counter regulate TGF-β activity during the response to renal injury are poorly understood.

Interestingly, another member of the TGF-β superfamily, BMP-7, inhibits TGF-β dependent signaling pathways.[6] The inhibitory effects of BMP-7 are mediated by the activation of SMAD1/5/8 proteins, which in turn suppress the activity of TGF-β dependent transcription factors and their ability to stimulate profibrotic gene expression.[7,8] Treatment with recombinant BMP-7 inhibits the development of renal injury in response to urinary obstruction and various other stimuli.[6–17]

While the renal protective effects of recombinant BMP-7 are well established, at our laboratory it was recently noted that endogenous BMP-7 activity is required for cessation of the TGF-β response along with the restoration of renal architecture and the resolution of fibrotic changes in the kidney that occur during the repair of obstruction induced renal injury.[8] The importance of BMP-7 in the repair of renal injury was further supported by studies at our laboratory and by others showing that treatment with recombinant BMP-7 can reverse the progression of chronic renal injury.[8–10]

Despite the biological importance of BMP-7 little is known about the molecular mechanisms that regulate endogenous BMP-7 activity and its renal protective functions. However, at our laboratory it was found that BMP-7 expression is up-regulated during kidney repair after acute obstruction induced renal injury.[7,8] Conversely, chronic renal injury in response to various stimuli, including urinary obstruction, is associated with loss of BMP-7 expression.[8,11–13,18] Along with the previous findings this suggests that loss of BMP-7 expression is a critical event during the pathogenesis of chronic renal injury that may lead to the suppression of BMP-7 dependent repair mechanisms and in part the persistent, inappropriate activation of TGF-β dependent profibrotic pathways.

In this study we delineated the molecular mechanisms that lead to loss of BMP-7 expression in the obstructed kidney and examined their potential importance for the treatment of obstructive uropathy and other conditions that lead to chronic renal injury.

## MATERIALS AND METHODS

### Unilateral Ureteral Obstruction

UUO was created in 8 to 10-week-old C57BL/6J mice by placing a microvascular clamp on the proximal ureter.[2] When indicated, mice were treated with 500 μg/kg TSA (Sigma®) daily by intraperitoneal injection. All procedures were approved by institutional review.

### Histology

Trichrome staining was done using the Accustain™ Masson trichrome staining kit. Immunofluorescence was performed using rabbit anti-BMP-7 and rabbit anti-type IV collagen (Abcam®), as previously described.[8] Tubular volume was quantified in samples stained for type IV collagen by digitally overlaying a grid on microscopy images and determining the percent of grid points located in the interstitial/tubular regions.[14]

### Collagen Quantification

Kidney samples were hydrolyzed and hydroxyproline was quantified by comparison to standards in a colorimetric reaction, as previously described.[19] Collagen content was calculated using the approximation that collagen contains approximately 14% (weight per weight) hydroxyproline. Values are expressed as a ratio to the dry tissue weight of the sample.

### BMP-7 Enzyme-Linked Immunosorbent Assay

Kidneys were pulverized in liquid nitrogen. They were homogenized in 100 mM tris-HCl (pH 7.4), 150 mM NaCl, 1% Triton®, 0.5% sodium deoxycholate, 1 mM ethylenediaminetetraacetic acid, 1 mM ethylene glycol tetraacetic acid and Complete Protease Inhibitor Cocktail (Roche, Mannheim, Germany). Samples were normalized to total protein content using the Pierce™ BCA Protein Assay Kit. BMP-7 levels were quantified using the BMP-7 ELISA Kit (R&D Systems®) according to product specifications.

### Reverse Transcription-PCR

Kidneys were pulverized in liquid nitrogen and homogenized in TRIzol®. RNA was isolated. RT-PCR was done using the SuperScript™ RT-PCR system with primers specific for BMP-7 (5′-GAAAACAGCAGCAGTGACCA-3′ and 5′-GCTCAGGAGAGGTTGGTCTG-3′), TGF-β1 (5′-CAAACGTCGGGGCGACCTGG-3′ and 5′-TGCTCCACCTTGGGCTTGCG-3′), α-SMA (5′-CCCTGAGACGCTGCTCCAGCTA-3′ and 5′-GGCATAGAGGGACAGCACAGCCT-3′), COLIα1 (5′-TGGTCCTGCCGGTCCTCCTG-3′ and 5′-ACACATTGGGGGTAGGAACA-3′), and GAPDH (5′-AACTTTGGCATTGTGGAAGG-3′ and 5′-ACACATTGGGGGTAGGAACA-3′). The relative intensity of PCR bands was quantified using ImageJ (http://rsbweb.nih.gov/ij/) and normalized as a ratio to GAPDH levels.

### Chromatin Immunoprecipitation

Chromatin immunoprecipitation was performed using the Imprint® Chromatin Immunoprecipitation Kit. DNA was sheared to 2 kb fragments by sonication.