Published in final edited form as:

J Urol. 2014 January ; 191(1): 242–252. doi:10.1016/j.juro.2013.06.110.

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

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Abstract

PURPOSE—Recombinant BMP-7 inhibits the pathogenesis of renal injury in response to a variety of stimuli. However, little is known about the molecular regulation of endogenous BMP-7 and its renal protective functions. This study examines the transcriptional regulation of *Bmp-7* and its role in the pathogenesis of renal injuries resulting from urinary tract dysfunction.

MATERIALS AND METHODS—Obstruction-induced renal injury was modeled *in vivo* in mice by unilateral ureteral obstruction (UUO) and *in vitro* in primary kidney cells by treatment with TGF- β , a pro-fibrotic cytokine that is elevated in the obstructed kidney.

RESULTS—UUO results in the loss of BMP-7 expression in conjunction with histone deacetylation and transcriptional repression of the *Bmp-7* promoter. The histone deacetylase (HDAC) inhibitor trichostatin A (TSA) stimulates *Bmp-7* expression in primary kidney cells. Furthermore, TSA inhibits the expression of TGF- β -dependent pro-fibrotic genes in a manner that is dependent upon BMP receptor signaling. These findings extend to the obstructed kidney *in vivo* where treatment with TSA restores the expression of *Bmp-7* along with the BMP-7-mediated suppression of TGF- β -dependent signaling pathways. Finally, the TSA-stimulated activation of the BMP-7 pathway ameliorates obstruction-induced renal injuries by preventing the disruption of renal architecture and the development of renal fibrosis.

CONCLUSIONS—Together, these findings demonstrate that the HDAC-dependent repression of *Bmp-7* transcription is a critical event during the pathogenesis of renal injury in obstructive uropathies. Accordingly, treatment with HDAC inhibitors represents a potentially effective strategy to restore BMP-7 expression and its renal protective functions during the treatment of obstructive uropathies.

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DISCLOSURE OF FUNDING SOURCES

This research was supported by funding from the NIH/NIDDK (1R01DK096177).

Keywords

Bone Morphogenic Protein 7; Fibrosis; Histone Deacetylases; Transforming Growth Factor Beta; Ureteral Obstruction

INTRODUCTION

Obstructive uropathies are a leading cause of pediatric kidney disease, accounting for 16.5% of kidney transplants in children.¹ While the kidney possesses innate repair mechanisms that are capable of restoring renal structure and function following acute injury due to urinary obstruction and other stimuli,^{2–4} the clinical manifestation of chronic renal injury stems in part from a dysregulated wound healing response that results in renal fibrosis.^{3, 4} At the molecular level, the activation of the TGF- β pathway has classically been described as a critical pathologic step in the development of renal fibrosis that promotes apoptosis, cellular de-differentiation, myofibroblast activation, and matrix protein synthesis.⁵ Given that these same processes are involved in injury repair,⁴ it is likely that TGF- β also plays a physiologic role in the early stages of injury repair but, when TGF- β activity is dysregulated, it instead promotes disease progression. Nonetheless, the mechanisms that counterregulate TGF- β activity during the response to renal injury are poorly understood.

Interestingly, another member of the TGF- β superfamily, BMP-7, has been demonstrated to inhibit TGF- β -dependent signaling pathways.⁶ The inhibitory effects of BMP-7 are mediated by the activation of the SMAD1/5/8 proteins which, in turn, suppress the activity of TGF- β -dependent transcription factors and their ability to stimulate pro-fibrotic gene expression.^{7, 8} Importantly, treatment with recombinant BMP-7 inhibits the development of renal injury in response to urinary obstruction and a variety of other stimuli.^{6–17} While the renal protective effects of recombinant BMP-7 are well established, our laboratory recently demonstrated that endogenous BMP-7 activity is required for the 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 injuries.⁸ The importance of BMP-7 in the repair of renal injuries is further supported by studies in our laboratory and by others which have demonstrated that treatment with recombinant BMP-7 is capable of reversing the progression of chronic renal injuries.^{8–10}

Despite the biologic importance of BMP-7, little is known about the molecular mechanisms that regulate endogenous BMP-7 activity and its renal protective functions. However, our laboratory found that BMP-7 expression is upregulated during kidney repair following acute obstruction-induced renal injury.^{7, 8} Conversely, chronic renal injury in response to a variety of stimuli, including urinary obstruction, is associated with the loss of BMP-7 expression.^{8, 11–13, 18} Along with the previously discussed findings, this suggests that the loss of BMP-7 expression is a critical event during the pathogenesis of chronic renal injuries that may lead to both the suppression of BMP-7-dependent repair mechanisms and, in part, the persistent and inappropriate activation of TGF- β -dependent pro-fibrotic pathways. In this study, we delineate the molecular mechanisms that lead to the loss of BMP-7 expression in

the obstructed kidney and examine their potential importance during the treatment of patients with obstructive uropathies and other conditions that lead to chronic renal injury.

MATERIALS AND METHODS

Unilateral Ureteral Obstruction

A unilateral ureteral obstruction was created in 8–10 week old C57BL/6J mice by placing a microvascular clamp on the proximal ureter.² When indicated, mice were treated with 500 µg/kg of trichostatin A (*Sigma*) daily by intraperitoneal injection. All procedures were approved by institutional review.

Histology

Trichrome staining was performed using the Accustain Masson's Trichrome Staining Kit (*Sigma*). Immunofluorescence was performed by 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 percentage 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% (w/w) hydroxyproline and expressed as a ratio to the dry tissue weight of the sample.

BMP-7 ELISA

Kidneys were pulverized in liquid nitrogen and homogenized in 100 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1% Triton, 0.5% sodium deoxycholate, 1 mM EDTA, 1 mM EGTA, and Complete Protease Inhibitor Cocktail (*Roche*). Samples were normalized according to total protein content using the BCA Protein Assay Kit (*ThermoScientific*). BMP-7 levels were quantified by using the BMP-7 ELISA Kit (*R&D Systems*) according to product specifications.

RT-PCR

Kidneys were pulverized in liquid nitrogen, homogenized in Trizol (*Invitrogen*), and RNA was isolated. RT-PCR was conducted by using the Superscript RT-PCR system (*Invitrogen*) and primers specific for BMP-7 (5'-GAAAACAGCAGCAGTGACCA-3'; 5'-GCTCAGGAGAGGT TGGTCTG-3'), TGF-β1 (5'-CAAACGTCGGGGCGACCTGG-3'; 5'-TGCTCCACCTTGGGC TTGCG-3'), α-smooth muscle actin (5'-CCCTGAGACGCTGCTCCAGCTA-3'; 5'-GGCATAG AGGGACAGCACAGCCT-3'), the α1 chain of type I collagen (5'-TGGTCCTGCCGGTCCT CCTG-3'; 5'-ACACATTGGGGGTAGGAACA-3'), or GAPDH (5'-AACTTTGGCATTGTGG AAGG-3'; 5'-ACACATTGGGGGTAGGAACA-3'). Relative intensities of PCR bands were quantified using ImageJ (*NIH*) analysis software and normalized as a ratio to GAPDH levels.

Chromatin Immunoprecipitation

Chromatin immunoprecipitation was performed using the Imprint ChIP kit (*Sigma*). DNA was sheared to 2 kb fragments by sonication. Immunoprecipitation was performed using either rabbit IgG or rabbit anti-acetyl-histone H3 (*Sigma*). PCR was performed using primers specific to the –407 to –151 bp region of the proximal *Bmp-7* promoter (5'-GTTTGTGCTGGTGCCCGCG-3'; 5'-GCTCTACGCGGATCCGGG-3'). Relative intensities of PCR bands were quantified using ImageJ (*NIH*) analysis software and normalized as a ratio to PCR products obtained from reactions performed on input DNA.

Culture of Primary Kidney Cells

Murine inner medullary collecting duct cells (IMCD-3) were obtained from *ATCC*. Cells were cultured in DMEM: F12 growth media supplemented with 10% FBS. When indicated, cells were treated with 100 nM trichostatin A (*Sigma*), 1 ng/mL TGF- β (*R&D Systems*), 10 ng/mL BMP-7 (*R&D Systems*), or 10 μ M dorsomorphin (*Sigma*).

Statistical Analysis

Statistical significance was analyzed by *t*-test or ANOVA with Bonferroni correction for multiple comparisons as appropriate. Data are presented as mean values $\pm$ standard deviation.

RESULTS

Obstruction-Induced Renal Injury is Associated with the Transcriptional Repression of Bmp-7 Expression

To examine the molecular regulation of BMP-7, we studied a murine model of obstruction-induced renal injury following unilateral ureteral obstruction (UUO) (Fig. 1A, 1B).² UUO results in a dramatic decrease in BMP-7 expression in a variety of cell types throughout the kidney (Fig. 1A). In quantifying the loss of BMP-7 expression, we found that UUO-induced renal injury is associated with an $88.1 \pm 11.1\%$ decrease in BMP-7 protein levels ($p < 0.005$) (Fig. 2A) along with a corresponding $83.1 \pm 12.8\%$ decrease in BMP-7 mRNA levels ($p < 0.01$) (Fig. 2B, 2C). Together, these data suggested the possibility that the loss of BMP-7 expression is mediated at the transcriptional level.

Accordingly, we sought to determine whether the loss of BMP-7 mRNA expression is associated with chromatin modifications in the regulatory regions of the *Bmp-7* gene. Indeed, we found that UUO results in a $63.0 \pm 8.5\%$ decrease in the acetylation of histone H3 proteins bound to the proximal *Bmp-7* promoter ($p < 0.005$) (Fig. 2D, 2E). This is a significant finding given that a number of transcriptional factors function by recruiting histone deacetylase (HDAC) complexes to target genes, a process that subsequently promotes chromatin condensation and transcriptional repression.²⁰

HDAC Proteins Regulate Bmp-7 Expression and the BMP-7-Mediated Suppression of TGF- β -Dependent Pro-Fibrotic Pathways In Vitro

To begin to investigate the role that changes in histone acetylation play in the regulation of BMP-7 expression, we examined the transcriptional regulation of *Bmp-7* in inner medullary

collecting duct cells (IMCD-3), a significant source of BMP-7 in the kidney.⁶ Subsequently, we found that treatment with the *pan*-HDAC inhibitor trichostatin A (TSA)²⁰ stimulates a 5.3 ± 1.2 fold increase in BMP-7 mRNA levels in IMCD-3 cells ($p < 0.005$) (Fig. 3A, 3B). This finding, along with those in Fig. 2D and Fig. 2E, demonstrates that *Bmp-7* expression is repressed by HDAC proteins.

We next sought to determine the importance of the HDAC-dependent regulation of *Bmp-7* expression in the renal protective functions of the BMP-7 pathway. To accomplish this, we studied an *in vitro* model of TGF- β -mediated renal fibrosis and used the suppression of TGF- β -dependent pro-fibrotic gene expression as a functional readout for BMP-7 activity. As previously demonstrated, treatment with TGF- β induces the expression of several pro-fibrotic genes that are central to the pathogenesis of renal injury including the $\alpha 1$ chain of type I collagen (*COL1a1*; a gene that encodes a protein that is a significant contributor to fibrosis)²¹ ($p < 0.005$) (Fig. 4A, 4B) and α -smooth muscle actin (*α -SMA*; a gene that encodes a protein that contributes to the differentiation of pro-fibrotic myofibroblasts)²² ($p < 0.005$) (Fig. 4A, 4C). Importantly, co-treatment with recombinant BMP-7 results in a $67.3 \pm 25.8\%$ decrease in *COL1a1* induction ($p < 0.005$) (Fig. 4A, 4B) and a $68.7 \pm 19.1\%$ decrease in *α -SMA* induction ($p < 0.005$) (Fig. 4A, 4C), demonstrating that the activation of the BMP-7 pathway is sufficient to suppress TGF- β -dependent pro-fibrotic gene expression in this model system.

In examining the effects of HDAC inhibition on the activity of the BMP-7 pathway, we found that treatment with TSA results in a 4.4 ± 1.5 fold increase in *Bmp-7* expression ($p < 0.005$) and that the stimulation of *Bmp-7* expression is not affected by co-treatment with TGF- β (Fig. 4A, 4D). In a manner similar to treatment with BMP-7, co-treatment with TSA results in a $73.7 \pm 21.9\%$ decrease in TGF- β -induced *COL1a1* expression ($p < 0.005$) (Fig. 4A, 4B) and a $60.0 \pm 13.6\%$ decrease in TGF- β -induced *α -SMA* expression ($p < 0.005$) (Fig. 4A, 4C). Given that TGF- β -dependent Smad-binding sites have previously been shown to be required for promoter activity in the *COL1a1* and *α -SMA* genes,^{21, 22} it is likely that the inhibitory effects of TSA treatment are mediated by the suppression of downstream steps in the TGF- β pathway. In support of this possibility, treatment with TSA has only minimal effects on the baseline expression of *COL1a1* (Fig. 4A, 4B), *α -SMA* (Fig. 4A, 4C), and endogenous *TGF- β 1* (Fig. 4A, 4E) in the absence of recombinant TGF- β .

We next sought to determine the requirement for BMP-7 activity in the ability of TSA to inhibit TGF- β -dependent pro-fibrotic gene expression. To accomplish this, we examined the effects of dorsomorphin, a pharmacologic inhibitor of BMP receptor activity,²³ on the inhibitory effects of TSA. As in Fig. 4, treatment with TSA stimulates *Bmp-7* expression ($p < 0.005$) and suppresses TGF- β -induced *COL1a1* ($p < 0.005$) and *α -SMA* ($p < 0.005$) expression (Fig. 5A–5D). However, when combined with dorsomorphin treatment, the inhibitory effects of TSA on TGF- β -induced *COL1a1* expression are reduced by $66.8 \pm 35.5\%$ ($p < 0.01$) (Fig. 5A, 5C) and on TGF- β -induced *α -SMA* expression are reduced by $77.9 \pm 17.1\%$ ($p < 0.05$) (Fig. 5A, 5D). Importantly, treatment with dorsomorphin does not affect TSA-stimulated *Bmp-7* expression (Fig. 5A, 5B) and has only minimal effects on the baseline expression of *COL1a1* (Fig. 5A, 5C), *α -SMA* (Fig. 5A, 5D), and endogenous *TGF- β 1* (Fig. 5A, 5E) in the absence of recombinant TGF- β . Together, these findings demonstrate

that the TGF- β -suppressing effects of HDAC inhibition are largely dependent upon stimulating the activity of the BMP-7 pathway.

HDAC Inhibition Restores BMP-7 Expression, Suppresses TGF- β Activity, and Ameliorates Renal Injury in Obstructive Uropathies

To extend these findings to the pathogenesis of chronic renal injury *in vivo*, we next sought to examine the effects of HDAC inhibition on BMP-7 expression in our murine model of obstructive uropathy. While UUO results in a significant decrease in BMP-7 protein levels, treatment with TSA restores BMP-7 expression in the obstructed kidney by stimulating a 4.0 ± 0.5 fold increase in BMP-7 protein levels ($p < 0.01$) (Fig. 6A). Importantly, these changes in BMP-7 expression at the protein level are paralleled by a 3.4 ± 1.9 fold increase in BMP-7 mRNA expression ($p < 0.01$) (Fig. 6B, 6C). Along with our findings in Fig. 2D and Fig. 2E, these results demonstrate that *Bmp-7* transcription is regulated by HDAC-dependent mechanisms *in vivo*.

Subsequently, we sought to determine whether the HDAC-dependent repression of *Bmp-7* expression plays a role in the molecular mechanisms that respond to renal injury. As shown in previous studies, UUO results in a significant upregulation in the expression of *TGF- β 1* ($p < 0.005$) (Fig. 6B, 6D) along with its downstream pro-fibrotic target genes *COL1a1* ($p < 0.005$) (Fig. 6B, 6E) and α -SMA ($p < 0.05$) (Fig. 6B, 6F) during the development of obstruction-induced renal fibrosis.⁵ Importantly, treatment with TSA not only restores BMP-7 expression in the obstructed kidney ($p < 0.01$) (Fig. 6A–6C), but also reduces *COL1a1* expression by $41.6 \pm 7.0\%$ ($p < 0.005$) (Fig. 6B, 6E) and α -SMA expression by $61.6 \pm 37.0\%$ ($p < 0.05$) (Fig. 6B, 6F). Furthermore, TSA treatment has only minimal effects on *TGF- β 1* expression (Fig. 6B, 6D), demonstrating that the inhibitory effects of HDAC inhibition are mediated by the suppression of downstream steps in the TGF- β pathway. Along with our prior findings (Fig. 4, 5), these results strongly suggest that the HDAC-dependent repression of *Bmp-7* expression contributes to the dysregulation of the TGF- β pathway following chronic renal injury.

Since the findings in Fig. 6 demonstrated that HDAC inhibition restores BMP-7 expression and the balance in TGF- β /BMP-7 signaling in the obstructed kidney, we next examined the therapeutic effects of treatment with HDAC inhibitors during the progression of chronic renal injury. While UUO results in the loss of BMP-7 expression in a variety of cell types in the obstructed kidney, treatment with TSA effectively targets each of these cell populations to restore BMP-7 expression (Fig. 7A). More importantly, treatment with TSA also markedly inhibits the development of UUO-induced renal injury. TSA treatment is associated with a $31.1 \pm 7.6\%$ decrease in total renal collagen content ($p < 0.05$) (Fig. 7A, 7B), a striking finding given that this corresponds to a 65.3% reduction in the increase in collagen expression above baseline levels following UUO. Furthermore, TSA treatment results in a $43.4 \pm 10.1\%$ decrease in the loss of tubular volume following UUO ($p < 0.01$) (Fig. 7A, 7C). Together, these findings demonstrate that the administration of pharmacologic HDAC inhibitors is an effective therapeutic approach not only to prevent the loss of BMP-7 expression in the injured kidney, but also to inhibit the development of renal

fibrosis and the disruption of renal architecture during the progression of chronic renal injury.

DISCUSSION

Over the last decade, BMP-7 has emerged as a critical renal protective protein that safeguards the kidney against a variety of stimuli that cause renal injury.^{6–17} However, the loss of BMP-7 expression occurs in obstructive uropathies and other conditions that lead to chronic renal injury.^{8, 11–13, 18} While the mechanisms that lead to the loss of BMP-7 expression have remained unclear, here we demonstrate in a mouse model of obstructive uropathy that the loss of BMP-7 expression occurs at the mRNA level in conjunction with histone deacetylation and transcriptional repression of the *Bmp-7* promoter (Fig. 2). Importantly, treatment with the *pan*-specific HDAC inhibitor TSA restores BMP-7 expression in the obstructed kidney (Fig. 6).

In examining the role that the HDAC-dependent repression of *Bmp-7* expression plays in the molecular mechanisms that respond to renal injury, we found that HDAC inhibition limits the pro-fibrotic potential of renal epithelial cells *in vitro* by suppressing the downstream steps in the TGF- β signaling pathway in a BMP-7-dependent manner (Fig. 4, 5). These signaling mechanisms (summarized in Fig. 8) also have considerable implications during the *in vivo* response to renal injury as demonstrated by the finding that treatment with TSA inhibits the expression of TGF- β -induced pro-fibrotic genes in the obstructed kidney (Fig. 6). We have previously published that BMP-7 is required for both the cessation of the TGF- β response and the repair of obstruction-induced renal injuries.⁸ The findings in this study strongly suggest that the HDAC-dependent repression of *Bmp-7* expression contributes to the persistent activation of the TGF- β pathway and the dysregulation of kidney repair following chronic renal injury.

Based on these findings, we have begun to examine the therapeutic potential of administering pharmacologic HDAC inhibitors as a means of limiting the progression of chronic renal injuries. Importantly, treatment with TSA significantly inhibits the development of renal fibrosis and the loss of renal architecture in response to urinary tract dysfunction (Fig. 7). While the suppression of TGF- β signaling is likely to account for many of the anti-fibrotic effects of TSA-stimulated BMP-7 expression, TGF- β -independent mechanisms likely also play an important role in inhibiting the pathogenesis of renal injury. We and others have previously shown that recombinant BMP-7 promotes the regeneration of renal architecture following injury^{8–10, 17} in a manner that recapitulates the requirement for BMP-7 during kidney development.²⁴ A possible role for these functions in the renal protective effects of HDAC inhibition is suggested by a recent study that identified an HDAC inhibitor as the leading candidate in a high throughput screen designed to identify compounds that stimulate regenerative cell populations within the kidney.²⁵

To fully realize the therapeutic potential of HDAC inhibitors, it will be necessary to translate these findings to *in vivo* models which more closely parallel the development of kidney disease following congenital urinary obstruction. While our study examined obstruction-induced renal injury in the mature kidney, surgical models of fetal/neonatal obstruction as

well as genetic models of congenital obstruction may provide useful tools for examining the importance of these biological pathways in the developing kidney.²⁶ Additionally, while our treatment regimen for the administration of TSA is supported by prior pharmacokinetic studies,²⁷ these protocols may be improved through further optimization. Finally, the use of isoform-specific HDAC inhibitors may provide a means of developing more specific and effective therapies for kidney disease.

CONCLUSIONS

In working towards the development of novel therapies for chronic kidney disease, it has become increasingly evident that the loss of BMP-7 is a critical molecular event that contributes to disease progression across a broad spectrum of etiologies.^{6–18} This study demonstrates that the loss of BMP-7 occurs by the HDAC-dependent repression of *Bmp-7* transcription in a murine model of obstructive uropathy. Furthermore, treatment with HDAC inhibitors is an effective therapeutic approach to restore BMP-7 expression and limit the progression of chronic renal injury in the obstructed kidney. The importance of these findings is accentuated by studies which have demonstrated the renal protective effects of HDAC inhibition in other settings.^{28–30} Although the mechanisms underlying these effects have remained unclear, this study demonstrates that the remarkable therapeutic potential of HDAC inhibitors is, in part, due to their ability to target the BMP-7 pathway. Given that HDAC inhibitors are already in clinical use, it is imperative to further examine the utility of these compounds in the treatment of obstructive uropathies and other conditions that lead to chronic kidney disease.

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A. **B.**

Figure 1. Obstruction-Induced Renal Injury Leads to the Loss of BMP-7 Expression

Mice ($n=5$) underwent either a sham operation or unilateral ureteral obstruction (UUO) for 7 days. Kidneys were analyzed by (A) (left) Masson's Trichrome staining (200x), (middle) Type IV Collagen immunofluorescence (green) with DAPI counterstaining (blue) (400x), (right) BMP-7 immunofluorescence (red) with DAPI counterstaining (blue) (200x), and (B) quantification of collagen content. * denotes $p<0.05$.

A. **B.**

C. **D.**

E.

Figure 2. The Loss of BMP-7 Expression is Mediated at the mRNA Level in Conjunction with Histone Deacetylation in the Proximal Bmp-7 Promoter

Mice ($n=3$) underwent either a sham operation or unilateral ureteral obstruction (UUO) for 7 days. Kidneys were analyzed by (A) BMP-7 ELISA, (B) RT-PCR for *Bmp-7*, *TGF- β 1*, and *GAPDH* (control), (C) quantification of *Bmp-7* expression from Fig. 2B, (D) chromatin immunoprecipitation with anti-acetylated-histone H3 or rabbit IgG (control) followed by PCR directed against the proximal region (–407 to –151 bp) of the *Bmp-7* promoter, and (E) quantification of histone acetylation in the *Bmp-7* promoter from Fig. 2D. ** denotes $p<0.01$; *** denotes $p<0.005$.

A. **B.**

Figure 3. HDAC Proteins Repress BMP-7 mRNA Expression in Primary Kidney Cells
 IMCD-3 cells ($n=3$ plates/sample) were treated with PBS (control) or 100 nM trichostatin A (TSA) for 24 hours. Cells were analyzed by (A) RT-PCR for *Bmp-7* and *GAPDH* (control) and (B) quantification of *Bmp-7* expression from Fig. 3A. *** denotes $p<0.005$.

A. **B.**

C.

D.

E.

Figure 4. HDAC Inhibition Suppresses the Activity of TGF- β -Dependent Pro-Fibrotic Pathways in Primary Kidney Cells

IMCD-3 cells ($n=3$ plates/sample) were serum starved for 24 hours, reintroduced to serum-containing medium, and treated with either PBS (control) or 1 ng/mL TGF- β and either PBS

(control), 10 ng/mL BMP-7, or 100 nM TSA for 24 hours, as indicated. Cells were analyzed by (A) RT-PCR for *Bmp-7*, *TGF- β 1*, *COL1a1*, *α -SMA* and *GAPDH* (control), (B) quantification of *COL1a1* expression from Fig. 4A, (C) quantification of *α -SMA* expression from Fig. 4A, (D) quantification of *BMP-7* expression from Fig. 4A, and (E) quantification of *TGF- β 1* expression from Fig. 4A. *ns* denotes $p>0.05$; * denotes $p<0.05$; ** denotes $p<0.01$; *** denotes $p<0.005$.

A. **B.**

C.

D.

E.

Figure 5. The TGF- β -Suppressing Effects of HDAC Inhibition Are Dependent Upon the Stimulation of BMP Activity

IMCD-3 cells ($n=3$ plates/sample) were serum starved for 24 hours, reintroduced to serum-containing medium, and treated with either PBS (control) or 1 ng/mL TGF- β , either PBS (control), 10 ng/mL BMP-7, or 100 nM TSA, and either PBS (control) or 10 μ M dorsomorphin (DM) for 24 hours, as indicated. Cells were analyzed by (A) RT-PCR for *Bmp-7*, *TGF- β 1*, *COL1a1*, *α -SMA* and *GAPDH* (control), (B) quantification of *Bmp-7* expression from Fig. 5A, (C) quantification of *COL1a1* expression from Fig. 5A, (D) quantification of *α -SMA* expression from Fig. 5A, and (E) quantification of *TGF- β 1* expression from Fig. 5A. *ns* denotes $p>0.05$; * denotes $p<0.05$; ** denotes $p<0.01$; *** denotes $p<0.005$.

A. **B.**

C. **D.**

E. **F.**

Figure 6. HDAC Inhibition Restores BMP-7 Expression and Suppresses TGF- β -Dependent Pro-Fibrotic Pathways Following Obstruction-Induced Renal Injury

Mice ($n=3$) underwent either sham operation or unilateral ureteral obstruction (UUO) for 7 days and were treated with either PBS (control) or 500 μ g/kg of trichostatin A (TSA). Kidneys were analyzed by (A) BMP-7 ELISA, (B) RT-PCR for *Bmp-7*, *TGF- β 1*, *COL1 α 1*, *α -SMA*, or *GAPDH* (control), (C) Quantification of *Bmp-7* expression from Fig. 6B, (D) Quantification of *TGF- β 1* expression from Fig. 6B, (E) Quantification of *COL1 α 1* expression from Fig. 6B, and (F) Quantification of *α -SMA* expression from Fig. 6B. *ns* denotes $p > 0.05$; * denotes $p < 0.05$; ** denotes $p < 0.01$; *** denotes $p < 0.005$.

A.

B. **C.**

Figure 7. HDAC Inhibition Ameliorates Obstruction-Induced Renal Injuries

Mice ($n=5$) underwent either sham operation or unilateral ureteral obstruction (UUO) for 7 days and were treated with either PBS (control) or 500 µg/kg of trichostatin A (TSA). Kidneys were analyzed by (A) (*left*) Masson's Trichrome staining (200x), (*middle*) Type IV Collagen immunofluorescence (green) with DAPI counterstaining (blue) (400x), (*right*) BMP-7 immunofluorescence (red) with DAPI counterstaining (blue) (200x), (B) quantification of collagen content, and (C) quantification of tubular volume. * denotes $p < 0.05$; ** denotes $p < 0.01$.

Figure 8. The HDAC-Dependent Transcriptional Repression of Bmp-7 Potentiates TGF-β-Mediated Renal Fibrosis in Obstructive Uropathies

The BMP-7 protein is a secreted cytokine that normally functions to stimulate gene transcription by binding to its receptor and activating the Smad1/5/8 transcription factors. The activation of the BMP-7 pathway also suppresses the pro-fibrotic TGF-β pathway by sequestering Smad4, a protein necessary for the transcription of many TGF-β-dependent genes.⁶ These counter-regulatory mechanisms are important for the suppression of TGF-β-dependent pro-fibrotic pathways during kidney repair.⁸ However, chronic urinary tract obstruction results in the HDAC-dependent suppression of *Bmp-7* transcription. The loss of BMP-7 results in the dysregulation of kidney repair along with the persistent and inappropriate activation of the TGF-β pathway. Importantly, treatment with pharmacologic HDAC inhibitors restores BMP-7 expression, suppresses TGF-β-dependent signaling pathways, and prevents renal fibrosis following chronic urinary tract obstruction.