Loss of Tubular Bone Morphogenetic Protein–7 in Diabetic Nephropathy

SHI-NONG WANG, JANINE LAPAGE, and RAIMUND HIRSCHBERG
Division of Nephrology, Harbor-UCLA Research and Education Institute, Inc., and UCLA, Torrance, California.

Abstract. Bone morphogenetic protein–7 (BMP7), a member of the transforming growth factor–β (TGF–β) superfamily of cytokines, is highly expressed in renal tubules and generally promotes maintenance of epithelial phenotype. It was examined whether, during the evolution of experimental diabetic nephropathy, the renal expression of BMP7 and BMP7 receptors declines, and the hypothesis that loss of BMP7 activity is profibrogenic in proximal tubular cells was tested. Moreover, in vitro studies in cultured proximal tubular cells were performed to examine putative mechanisms that cause these changes. At 15 wk of streptozotocin-induced diabetes, renal expression of BMP7 is declined by about half, and it decreased further by 30 wk to <10% of timed controls. Renal expression of the high-affinity BMP type II receptor and the type I receptor Alk2 (activin receptor–like kinase-2) decreased. Alk3 tended to decrease, but Alk6 remained unchanged. During the evolution of diabetic nephropathy, the secreted BMP antagonist gremlin increased substantially. In cultured tubular cells, TGF–β reduced BMP7 and Alk3 expression and increased gremlin but did not interrupt BMP7-induced activation of smad5 or Erk1 and -2. In contrast, BMP7 did not alter TGF–β expression. Neutralization of endogenous BMP7 in cultured proximal tubular cells raised the expression of fibronectin and tended to increase collagen α1 III mRNA levels. In conclusion, in experimental diabetic nephropathy, renal tubular BMP7 and some of its receptors decreased and gremlin, a secreted BMP antagonist, increased. Some, but not all, of these changes are explained by increased TGF–β. The loss of BMP7 activity per se is profibrogenic in tubular cells.

Diabetic nephropathy is the most prevalent cause for end-stage renal disease in the United States and the Western world. In this disease, tubular injury and progressive interstitial fibrosis contribute significantly to renal failure and predict progression to end-stage renal disease (1–3). The tubular epithelium appears to play key roles in regulating onset and progression of interstitial fibrosis in diabetic nephropathy. Relatively early during the progression of diabetic nephropathy, renal tubules express increased amounts of profibrogenic transforming growth factor–β (TGF–β), which predates onset of interstitial fibrosis. This cytokine is perhaps a major biologic signal, although nonexclusive, that regulates a switch in tubular cell activities toward a profibrogenic phenotype (4,5). Bone morphogenetic protein–7 (BMP7) may function as an opponent to these changes.

BMP7 (formerly called osteogenic protein-1 or OP1) is a homodimeric member of the BMP subfamily within the TGF–β superfamily of cysteine-knot cytokines (6,7). In the adult organism, BMP7 is primarily expressed in kidney tubules, and lesser amounts are found in ovary, nonpregnant endometrium, and growth plates of cartilage (8,9).

BMP7 receptors are type I/II heterodimeric serine/threonine kinase receptors (10). Several different type I receptors, namely the activin receptor–like kinases (Alk) Alk2 (activin receptor I), Alk3 (BMP receptor Iα), and Alk6 (BMP receptor Iβ) associate with the BMP type II receptor (BMPRII). Receptor activation induces intracellular recruitment and serine/threonine phosphorylation of smad substrates. Smad1, -5, and -8 are BMP-restricted smads, and smad1 and/or -5 are signaling substrates for BMP7 in different cell types (10–12). TGF–β–restricted smad2 and -3 are not activated by BMP7.

The bioactivity of BMP7, like that of other TGF–β superfamily members, is further regulated by a series of secreted, extracellular proteins that bind and neutralize peptide activity. Secreted BMP7 antagonists include noggin, follistatin, and gremlin (10,13–17).

We tested the hypothesis that loss of BMP7 activity is profibrogenic in proximal tubular cells. Moreover, studies were performed to examine whether, during the evolution of experimental diabetic nephropathy, renal tubular levels of BMP7 decrease and the expression of BMP7 receptors and secreted antagonists changes in favor of reduced BMP7 bioactivity. In vitro studies were performed to examine potential mechanisms of these changes.

Materials and Methods

In Vivo Studies

Initial observations of immunohistologic expression of BMP7 were made in rats with streptozotocin-induced diabetes (n = 5) and timed controls (n = 5) at 30 wk. These animals had been prepared princi-
pally for other studies that have been reported elsewhere (18). Additional studies were performed in diabetic rats and timed controls (n = 5) at 15 wk. In these latter animals, in addition to immunohistologic studies, renal BMP7 mRNA levels, as well as mRNA levels of BMP7 receptors and antagonists and urine BMP7, were also examined.

Sprague-Dawley rats were made diabetic with a single intravenous injection of streptozotocin, 65 mg/kg (Sigma, St. Louis, MO). At 2 d, glucose was measured in tail blood with a glucometer, and animals with serum glucose levels ≥300 mg/dl were included. Rats were given NPH insulin subcutaneously, to maintain serum glucose at 300 to 350 mg/dl. Control animals did not receive streptozotocin or insulin.

In the first series, animals were killed at 30 wk. Kidneys were removed and ~1-mm coronal sections were fixed in 4% paraformaldehyde and embedded in paraffin. In the second series of rats, at 15 wk, urine was collected on ice for 12 h to determine BMP7 excretion. Kidneys were removed, and ~1-mm coronal sections were fixed and embedded; total RNA was extracted with RNA-Stat-60 (Tel Test, Friendswood, TX) from aliquot kidney sections.

Immunohistology for BMP7

Deparaffinized 5-μm sections were quenched, blocked, and incubated with anti-BMP7 antibody (1:200, Santa Cruz Biotechnology, Santa Cruz, CA) and biotin-labeled second antibody and visualized with streptavidin-horseradish peroxidase (HRPO) and a substrate reaction. For control, the primary antibody was replaced with nonimmune serum. Sections were lightly counterstained with hematoxylin.

Slides were examined with light microscopy, and BMP7-positive tubules were counted by use of a point-grid. Quantitative data were derived by dividing the point counts on BMP7-positive tubule cross-sections by the number of points on any tubule cross-section multiplied by 100, to express results as a percentage (19). In addition, immunohistology for TGF-β was also performed.

Urinary BMP7

Urine aliquots corresponding to 5% of excretion from three control and four diabetic rats, respectively, were diluted with sterile water and concentrated in spin concentrators (Millipore, Bedford, MA). Retentates were taken up in nonreducing sodium dodecyl sulfate–polyacrylamide gel electrophoresis sample buffer, electrophoresed in 15% gels, and transferred onto nitrocellulose. Membranes were blocked and blotted successively with anti-BMP7 (1:1000), biotinylated second antibody, and HRPO-streptavidin. Bands were visualized with chemiluminescence (Amersham, Arlington Heights, IL).

mRNA Levels Encoding BMP7, Alk2, Alk3, Alk6, BMPRII, Gremlin, Follistatin, and Noggin

mRNA levels encoding BMP7, BMP receptors, and BMP antagonists were measured by quantitative reverse transcription–PCR (RT-PCR). Random-primed first-strand cDNA was synthesized from RNA with a commercial procedure (Boehringer Mannheim, Indianapolis, IN). Aliquots of cDNA were amplified with specific primers for each of the mRNA species (Table 1), and 18S cDNA was coamplified as

### Table 1. Reverse transcriptase–PCR primers

<table>
<thead>
<tr>
<th>mRNA, Species</th>
<th>Sense Primer</th>
<th>Antisense Primer</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMP7</td>
<td>5′-CCTTTGGGCGACGCCTGGCAGGAC-3′</td>
<td>5′-GTCCAACTAGGCTGCGCAGCT-3′</td>
</tr>
<tr>
<td>Alk2</td>
<td>5′-TCTGTGCTAATGATGATGGCTTCCTCC-3′</td>
<td>5′-TTCTTGGCAGTCCAGGGAAGGATTTC-3′</td>
</tr>
<tr>
<td>Alk3</td>
<td>5′-TTCGGATTGTGCTGTCCATA-3′</td>
<td>5′-AACCTTGGGTCATGGGGAACC-3′</td>
</tr>
<tr>
<td>Alk6</td>
<td>5′-TTATCTCTGCTGCGTCCTGTG-3′</td>
<td>5′-CTTTACATCTCGGGATTCAAC-3′</td>
</tr>
<tr>
<td>BMPRII</td>
<td>5′-CAGGTATAAAAAGAGAAGGACGCG-3′</td>
<td>5′-TCTTCTATGTCCCTCATAAGAAGGG-3′</td>
</tr>
<tr>
<td>Gremlin</td>
<td>5′-AGGTTCCCAAGGAGCCATTC-3′</td>
<td>5′-CTGGACATCGAATGCTAGA-3′</td>
</tr>
<tr>
<td>Follistatin</td>
<td>5′-AAAGAGGCACTGTGAGAGACCGTGA-3′</td>
<td>5′-GATATGCAACGGGCACTGCTT-3′</td>
</tr>
<tr>
<td>Noggin</td>
<td>5′-ACGGCGGAGGACGAAGGCAGCC-3′</td>
<td>5′-CAATGGATCGTGGCCAGCAAG-3′</td>
</tr>
<tr>
<td>Collagen α1 III</td>
<td>5′-TTGGTACACCGGAAGCATTATCAGATAA-3′</td>
<td>5′-TGGATCAAAACATTTCTCAGCTATTGG-3′</td>
</tr>
</tbody>
</table>

* BMP, bone morphogenetic protein; ALK, activin receptor–like kinase; BMPRII, BMP type II receptor.
the internal standard in each tube with specific primers and a compatimer (Ambion, Inc., Austin, TX). Temperature cycling, cycle number, and compatimer concentrations were optimized for each mRNA species. PCR products were electrophoresed in 4% agarose gels that contained 0.5 μg/ml of ethidium bromide. Resolved gels were photographed with Polaroid negative film (Polaroid, Cambridge, MA). Bands were scanned by densitometry, and densitometric units were expressed relative to the 18S mRNA band in the same sample as a percentage of control.

**In vitro Studies**

Experiments were performed by use of murine proximal tubular cells (mPTC) that were derived from S3 segments of mouse proximal tubules by microdissection (18,20). Cells were grown in Petri dishes or 6-well plates in Dulbecco’s modified Eagle’s medium/F12 that contained insulin, transferrin, selenite, and 10% fetal calf serum. At ~95% confluence, cells were growth arrested in serum-free medium that contained 0.1% bovine serum albumin for 24 h before individual experiments.

**Effects of Neutralization of BMP7 on Matrix Protein Expression in mPTC**

Because increased expression of interstitial extracellular matrix proteins has previously been shown to be an abnormal function of tubular cells in diabetic nephropathy, experiments were performed to examine whether neutralization of BMP7 activity in mPTC is associated with changes in fibronectin and/or collagen α3,III expression. BMP7 was neutralized with a recombinant fusion protein that consisted of the high-affinity extracellular domain of Alk6 fused to the Fc region of IgG. Confluent, arrested cells in 6-well plates were incubated without (control) or with the Alk6/Fc chimera protein (R&D Systems, Minneapolis, MN), 2 μg/ml, for 72 h, and media were changed at 24 and 48 h to maintain high levels of active Alk6/Fc-chimeras, (n = 6 each). Cells were then washed, and total RNA was extracted from each well. mRNA encoding fibronectin and collagen α3,III were measured by quantitative RT-PCR.

**BMP7 Cell Signaling in mPTC**

Studies were performed to examine the preferred, BMP7-activated smad substrate in mPTC and to examine whether BMP7 activates extracellular signal-regulated kinase-1 and/or -2 (Erk1/2).

**Smad Phosphorylation**

Confluent, growth-arrested proximal tubular cells in a 6-well plate were incubated with 1 nM rhBMP7 (kind gift from Kuber Sampath, Creative Biomolecules, Boston, MA) (dissolved in 24 mM Na acetate [pH 4.5] containing 1% Nonidet P-40, 0.5% Na-deoxycholate, 0.1% sodium dodecyl sulfate, 100 μg/ml phenylmethylsulfonyl fluoride, 30 μg/ml aprotinin [Sigma No. A6279], 100 μM of Na-orthovanadate, 2 μM of leupeptin, and 4 μM of pepstatin A), 1 ml/well. Lysates were cleared by centrifugation. Immunoprecipitations were performed with an antibody that recognizes smad1 and -5 (Santa Cruz Biotechnology) and protein G-plus-agarose overnight. Immunoprecipitates were washed four times with lysate buffer, and pellets were electrophoresed in a 10% minigel. Separated proteins were transferred onto nitrocellulose and immunoblotted with anti-phospho-serine antibody (1:300, Zymed, South San Francisco, CA), biotinylated second antibody, and HRPO-streptavidin. Bands were visualized by chemiluminescence and captured on x-ray film.

In separate experiments, confluent, growth-arrested mPTC in 10-cm culture plates were phosphate-depleted with P-free medium for 30 min and then metabolically labeled with 200 μCi/ml 32P-orthophosphoric acid (ICN, Irvine, CA) for 75 min. Subsequently, 1 nM rhBMP7 or BMP7-free diluent (control) were added for a further 45 min. Cells were washed twice with ice-cold medium and lysed. Lysates were immunoprecipitated with anti-smad1/5 antibody and electrophoresed, and the dried gel was autoradiographed. In a parallel experiment, in cells that were not P-labeled, immunoprecipitates underwent Western blot analysis with either specific anti-smad1 or anti-smad5 antibody (Santa Cruz Biotechnology).

**Erk Phosphorylation**

TGF-β has been reported to activate Erk1 and -2 in certain cells, including epithelial cells (21). Hence, studies were performed to examine whether BMP7 alters TGF-β-induced Erk phosphorylation. In these experiments, mPTC in 6-well plates were incubated with rhTGF-β1 (R&D Systems) or rhBMP7, or both, each at 1 nM, for 30 min. Cells were washed twice with ice-cold medium. Equal aliquots of cell lysates were electrophoresed in 12% sodium dodecyl sulfate–polyacrylamide gel electrophoresis gels and transferred onto nitrocellulose. Membranes underwent Western blot analysis either with antibody specific for phosphorylated Erk or with anti-Erk antibody (Santa Cruz Biotechnology) and HRPO-labeled second antibody, and bands were visualized by chemiluminescence.

**Effects of BMP7 on TGF-β Expression in mPTC**

Cells that had been grown to confluence in 6-well plates and that were growth arrested were incubated with or without 1 nM rhTGF-β1 for 24 h (n = 6 each). Total RNA was extracted, and mRNA encoding BMP7, Alk2, Alk3, gremlin, or follistatin were measured by quantitative RT-PCR, as described above.

**Effects of BMP7 on TGF-β Expression in mPTC**

To examine the question of whether BMP7 reduces TGF-β expression in cultured proximal tubular cells, mPTC in 6-well plates were incubated with the peptide at 500 pM and 5 nM for 24 h (n = 6 each). Expression of TGF-β was measured by quantitative RT-PCR.

**Statistical Analyses**

Data are expressed as mean ± SEM. Statistical comparisons were made by ANOVA, followed by the Newman-Keuls Multicomparison test with the use of Stat-Most 2.5 software (Data Most Corporation, Salt Lake City, UT). P < 0.05 was defined as statistically significant.

**Results**

**In Vivo Findings**

**Tubular BMP7 Levels Progressively Decrease during the Evolution of Diabetic Nephropathy.** In normal rats, BMP7 is expressed immunohistologically lightly in some but not all cortical proximal tubular cross-sections and more intensely in straight (S3) portions in the corticomedullary junction. Distal convoluted tubules stain strongly, as do collecting ducts. This pattern gives rise to the intense staining of medullary rays seen at low magnification (Figure 1A). Glomeruli are negative for BMP7. At 15 wk of diabetes, tubular BMP7 is decreased
substantially, and many tubular segments that express the peptide in normal rats have become negative (Figure 1, A and B). Subsequently, BMP7 expression decreases further and virtually disappears by 30 wk (Figure 1, A and B).

The decrease in tubular BMP7 observed immunohistochemically in diabetic rats at 15 wk is associated with decreased urinary excretion of the protein (Figure 2). Moreover, renal BMP7 mRNA levels are also decreased at 15 wk of diabetic rats compared with those of controls (Figure 3).

In contrast to controls, where immunoreactive TGF-β is only found in glomerular arterioles but not in any other glomerular or in any tubular location, tubular expression of TGF-β is seen in proximal as well as distal tubular segments at 15 wk, and more so at 30 wk, of diabetes (data not shown), as has also been described by other investigators (22–25).

**Renal BMP7 Receptors Tend to Decrease in Diabetic Nephropathy.** mRNA levels encoding Alk2, Alk3, and Alk6, the BMP-type I receptors that bind BMP7 with high affinity, and BMPRII mRNA levels were examined in control and diabetic rat kidney. Renal Alk2 and BMPRII mRNA levels were significantly decreased at 15 wk of diabetes, compared with those in timed controls ($P < 0.05$), and Alk3 and Alk6 mRNA tended to be reduced, albeit not significantly (Table 2).

**Secreted BMP Antagonists.** Noggin mRNA is not found in normal or diabetic rat kidneys but is abundantly expressed in rat liver. Follistatin is expressed in both control and diabetic rat kidney, and mRNA levels at 15 wk were similar in both groups.

![Figure 1](image1.png)  
*Figure 1.* Immunoreactive bone morphogenetic protein-7 (BMP7) in control and diabetic (DM) rat kidney. (A) Representative microphotographs of a control and of coronal sections from diabetic rat kidneys at 15 and 30 wk, respectively. (B) BMP7-positive tubule cross-sections were counted by use of a point-grid method and are expressed as a percentage. *$P < 0.05$. Magnification, $\times 100$.  

![Figure 2](image2.png)  
*Figure 2.* Western blot analysis of urinary BMP7 in control (Co) and DM rats at 15 wk. Five percent of urine collected within 12 h was concentrated and analyzed under nonreducing conditions.

![Figure 3](image3.png)  
*Figure 3.* Renal BMP7 mRNA levels in Co and DM rat kidney at 15 wk. *$P < 0.05$. 

![Figure 4](image4.png)  
*Figure 4.*
In contrast, compared with controls, renal gremlin mRNA levels were significantly increased at 15 wk of diabetes (Table 2).

**In Vitro Findings**

**Neutralization of Endogenous BMP7.** Incubation of mPTC with an Alk6/Fc chimera protein that binds BMP7 with high affinity and neutralizes its bioactivity significantly increased the expression of fibronectin by ~35% ($P < 0.05$, Figure 4). Collagen $\alpha_{1}$III mRNA levels tended to increase, albeit not significantly (Figure 4).

**BMP7 Signaling in Proximal Tubular Cells.** Immunoprecipitation of lysates from mPTC with an antibody that recognizes smad1 and -5 demonstrates a single band upon Western blot analysis of precipitates with anti-phospho-serine antibody. This band is enhanced in cells exposed to BMP7, compared with controls (lane 3 in Figure 5A). Serine phosphorylation of this ~50 kD smad protein is not increased in cells exposed to equimolar TGF-β (lane 2 in Figure 5A). Moreover, TGF-β does not alter the BMP7-induced phosphorylation (lane 4 in Figure 5A). Immunoprecipitation of $^{32}$P-labeled cell lysates and Western blot analysis confirms that BMP7 phosphorylates either smad1 or -5 in proximal tubular cells (Figure 5B). In Western blot analysis, the phosphorylated band reacts with anti-smad5 (Figure 5C) but not anti-smad1 (not shown). These studies indicate that the exclusive or preferred smad signaling substrate for BMP7 in proximal tubular cells is smad5. Moreover, phosphorylation of smad5 by BMP7 is not inhibited by coincubation with TGF-β.

BMP7 does not antagonize TGF-β-induced phosphorylation of Erk1/2. In fact, in mPTC, BMP7 strongly activates Erk1 and -2 (Figure 6). Coincubation of cells with BMP7 and TGF-β causes additive Erk phosphorylation (Figure 6). Neither TGF-β nor BMP7 increase Erk1/2 protein levels (Figure 6).

**TGF-β Down-Regulates BMP7 and Modifies Expression of BMP7 Receptors and Antagonists.** In cultured proximal tubular cells, rhTGF-β reduces the expression of BMP7 to 49 ± 3% of control levels ($P < 0.05$). TGF-β also significantly down-regulates expression of Alk3 in proximal tubular cells to 51 ± 8% of control levels ($P < 0.05$) but does not affect Alk2 mRNA levels. Alk6 mRNA could not be detected in mPTC. In proximal tubular cells, follistatin mRNA levels were similar in controls and in cells that were incubated with TGF-β. Gremlin mRNA is up-regulated by TGF-β 2.4 ± 0.2-fold compared with control levels ($P < 0.05$).

**BMP7 Does Not Affect TGFβ Expression.** Although TGF-β down-regulates BMP7 mRNA levels in tubular cells, the reverse experiment is negative. rhBMP7 at either 0.5 or 5 nM does not affect expression of TGF-β.

**Discussion**

Renal interstitial fibrosis is a universal hallmark of progressive chronic renal failure. Different mechanisms may regulate the accumulation of extracellular matrix proteins that form interstitial scars in varying renal/glomerular diseases, but abnormal function of tubular epithelial cells is a major contributing factor (5,18,26). Diabetic nephropathy is the most com-
mon single cause for chronic renal failure and end-stage renal disease. In this disease, tubular cells produce increased amounts of extracellular matrix proteins and undergo a number of other phenotypic changes that contribute to the onset and progression of interstitial fibrosis. Early in the course of diabetic nephropathy, tubular cells begin to express TGF-β, which is perhaps, in part, induced by chronic hyperglycemia as well as by other less well-understood mechanisms (4). This cytokine raises the expression of interstitial extracellular matrix proteins in tubular cells. Another member of this superfamily of cytokines, BMP7, appears to be antifibrogenic and to oppose some of the profibrogenic actions of TGF-β. Indeed, reduction of BMP7 activity in vitro by neutralization with an Alk6/Fc chimera protein raises the expression of fibronectin and tends to increase collagen type III expression. Both of these proteins contribute to accumulating interstitial extracellular matrix in renal interstitial fibrosis in diabetic nephropathy, although the quantitative contribution of reduced BMP7 activity in vivo remains unclear. These findings suggest that endogenous BMP7 has antifibrogenic activities and, in this respect, counterbalances TGF-β activities. Very recently, several different investigators demonstrated that exogenously administered rh-BMP7 decreases interstitial fibrosis and progressive renal failure in various rat models of chronic progressive interstitial fibrosis, which further illustrates the antifibrogenic activity of this cytokine (27–30).

Renal tubular BMP7 expression decreases early in the course of experimental diabetic nephropathy; subsequently, BMP7 disappears almost completely from the kidney. Furthermore, other regulators of BMP7 bioactivity are also changed during the evolution of experimental diabetic nephropathy. These include decreased renal expression of BMPRII and of some of the type I receptors and increased expression of secreted BMP antagonists. Overall, these findings provide evidence that, in addition to reduced BMP7 expression and protein levels, its biologic activity in the kidney is further reduced at the receptor and antagonist level.

These changes in renal BMP7 levels and the expression of its receptors and antagonists tend to coincide with the expression of TGF-β, giving rise to the possibility that TGF-β downregulates BMP7, and this may be a major mechanism of the action of TGF-β. Alternatively, loss of BMP7 could trigger the increase in TGF-β expression during the evolution of diabetic nephropathy.

In cultured tubular cells, as shown in this study, TGF-β decreases BMP7 expression, which suggests that a rise in tubular TGF-β levels during the evolution of diabetic nephropathy contributes causally to the loss of BMP7. The contrary, BMP7-induced suppression of TGF-β, which would suggest that the loss of BMP7 may cause a rise in TGF-β, is unlikely, given that the respective experiment in cultured tubular cells is negative. TGF-β also reduces or tends to reduce the expression of BMP7 type I and II receptors in cultured tubular cells. This may suggest that TGF-β causes respective changes in renal receptor expression during the evolution of diabetic nephropathy in vivo.

Only one of the three BMP antagonists, gremlin, increases in diabetic rat kidneys, as has recently been shown by other investigators (31), as well as in this study. The present in vitro findings suggest that TGF-β causes or contributes to the increase in gremlin. Increased gremlin will likely contribute to a further decrease in BMP7 bioactivity beyond that caused by the decreased expression of the protein itself, because it neutralizes BMP7 activity by binding the protein in the extracellular environment (13,15).

In proximal tubular cells, BMP7 activates smad5 but apparently not (or only minimally) smad1. The current experiments have also shown that TGF-β does not antagonize the BMP7-induced serine phosphorylation of smad5. The specificity of BMP signals appears to be determined, in part, by the type I receptors (10). High-affinity BMP7 type I receptors that are expressed in tubular cells include Alk2 and -3, and activation

### Table 2. mRNA levels encoding BMP receptors and secreted BMP7 antagonists at 15 wk in diabetic and control rat kidney

<table>
<thead>
<tr>
<th>Control</th>
<th>1.00 ± 0.10</th>
<th>1.00 ± 0.11</th>
<th>1.00 ± 0.08</th>
<th>1.00 ± 0.05</th>
<th>Not detected</th>
<th>1.00 ± 0.08</th>
<th>1.00 ± 0.08</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic nephropathy</td>
<td>0.72 ± 0.09</td>
<td>0.85 ± 0.06</td>
<td>0.94 ± 0.06</td>
<td>0.79 ± 0.07</td>
<td>Not detected</td>
<td>0.96 ± 0.07</td>
<td>1.47 ± 0.10</td>
</tr>
</tbody>
</table>

**Probability**

|            | P < 0.05 | NS | NS | P < 0.05 | NS | P < 0.05 |

**a** n = 5 each. **b** Noggin is not expressed in rat kidney but is found abundantly in rat liver.

---

**Figure 6.** Phosphorylation of extracellular signal–regulated kinases 1 and -2 (Erk1/2) in murine proximal tubular cells. Cells were incubated without (control, Con) or with 1 nM BMP7, 1 nM TGF-β, or both (B+T). Aliquots of cell lysates underwent Western blot analysis with an antibody specifically that recognizes tyrosine-phosphorylated Erk1/2 (p-Erk1/2) or anti-Erk1/2 antibody (Erk1/2). Both, BMP7 as well as TGF-β, induce phosphorylation of Erk1 and -2. The effects of each of the two cytokines on Erk phosphorylation appears to be additive. Levels of Erk1/2 proteins are not changed.
of smad5 can be mediated through both of these type I receptors as well as through Alk6 (10–12).

BMP7 rapidly phosphorylates Erk1 and -2 in proximal tubular cells, as shown in these experiments (Figure 6). TGF-β also activates Erk1 and -2 in proximal tubular cells, as shown in this study (Figure 6) as well as by different investigators in other cell types (21). Activated Erk inhibits nuclear translocation of smad2 and -3 by phosphorylation in the linker region and, hence, reduces these TGF-β signals (32). One mechanism by which BMP7 could inhibit TGFβ-induced increased production of extracellular matrix (33) could be the activation of Erk1/2 by BMP7.

In summary, endogenous BMP7 prevents increased expression of interstitial extracellular matrix proteins in proximal tubular cells, and neutralization of BMP7 is in itself a profibrogenic event. During the evolution of experimental diabetic nephropathy, tubular BMP7 expression and expression of BMP7 receptors decreases, and secreted BMP antagonists increase. Some of these changes are induced by TGF-β.

Acknowledgments

The work in this article was supported by grants from the Juvenile Diabetes Foundation. Dr. Shi-Nong Wang was supported by a fellowship grant from the National Kidney Foundation of Southern California. Parts of the findings in this paper were presented in abstract form during the Annual Meeting of the American Society of Nephrology, Toronto, Canada, October 2000 (J Am Soc Nephrol 11: 520A and 11: 655A, 2000).

References


Access to UpToDate on-line is available for additional clinical information at http://www.jasn.org/