BMP7 antagonizes TGF-β-dependent fibrogenesis in mesangial cells

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Wang, Shinong and Raimund Hirschberg. BMP7 antagonizes TGF-β-dependent fibrogenesis in mesangial cells. Am J Physiol Renal Physiol 284: 1006–1013, 2003; 10.1152/ajprenal.00382.2002.—Exogenous administration of recombinant human bone morphogenetic protein (BMP)-7 was recently shown to ameliorate renal glomerular and interstitial fibrosis in rodents with experimental renal diseases. We tested the hypothesis that BMP7 functions by antagonizing profibrogenic events that are induced by transforming growth factor (TGF)-β in cultured mesangial cells. Incubation of murine mesangial cells with TGF-β (50–200 pM) increased cell-associated collagen type IV and fibronectin, soluble collagen type IV, thrombospondin, and connective tissue growth factor (CTGF). Coincubation with recombinant human BMP7 (200 pM) reduced the increase of these ECM proteins and CTGF. The changes in collagen type IV and fibronectin proteins occurred without concomitant changes in collagen type α1IV and fibronectin mRNA levels, suggesting that TGF-β and BMP7 act primarily by affecting ECM protein degradation. Indeed, TGF-β decreases the levels and activity of matrix metalloprotease (MMP)-2, the major metalloprotease that is secreted by mesangial cells. Moreover, BMP7 inhibits TGF-β-induced activation of MMP2. Because TGF-β reduces the activity of MMPs through increasing plasminogen activator inhibitor (PAI)-1, we tested whether BMP7 interferes with this TGF-β effect. BMP7 reduces, by about two-thirds, the activation of a PAI-1 promoter/luciferase reporter in cells stably transfected with this construct. The findings from these studies indicate that BMP7 reduces TGF-β-induced ECM protein accumulation in cultured mesangial cells primarily by maintaining levels and activity of MMP2 partially through prevention of TGF-β-dependent up-regulation of PAI-1.

bone morphogenetic protein 7; transforming growth factor-β; kidney fibrosis; matrix metalloprotease-2; plasminogen activator inhibitor-1

RENEAL FIBROSIS, i.e., ACCUMULATION OF ECM proteins in glomeruli and renal interstitium, is the hallmark of most advanced chronic renal diseases. Progressive fibrosis is the result of an imbalance between synthesis and degradation of ECM proteins. Several cytokines contribute to increased matrix accumulation, but transforming growth factor (TGF)-β has evolved as the single most important profibrogenic mediator in renal diseases.

Recent studies showed that another member of the TGF-β superfamily of cysteine-knot cytokines, bone morphogenetic protein (BMP)-7, can prevent or reduce the progression of renal fibrosis in animals with experimental renal diseases (7, 12, 13, 16, 18, 20). Morrissey and associates (18) showed that exogenously administered recombinant human (rh)BMP7 may even resolve, at least partially, early stages of established glomerular and interstitial fibrosis in experimental diabetic nephropathy.

BMP7 plays major roles during embryonic development, but in adult organisms expression of BMP7 and its receptors is retained in only few tissues, most prominently in the kidney (3, 4, 22, 29). Its functions in adult kidney are presently unknown but may include promotion of differentiated epithelial phenotype in tubular cells (7, 8, 31). Moreover, BMP7 may counteract some of the profibrogenic actions of TGF-β. Consistent with this latter hypothesis are previous findings indicating that a decrease in renal BMP7 expression precedes the onset of glomerular sclerosis and interstitial fibrosis in experimental obstructive nephropathy and diabetic nephropathy (12, 29).

The present studies were performed to examine the hypothesis that BMP7 reduces ECM accumulation in mesangial cells and antagonizes (some of the) profibrogenic events that are induced by TGF-β.

MATERIALS AND METHODS

Mouse mesangial cells derived from a SV40-expressing mouse were obtained from ATCC, Manassas, VA (17). Cells were grown to >90% confluence in DMEM/F-12 (3:1) containing 14 mM HEPES and 5% FCS. Before each experiment, cells were incubated in serum-free medium containing 0.1% BSA. In individual experiments, cells were incubated for 24 to 72 h with rhTGF-β (Biosource, Camarillo, CA), 50, 100, or 200 pM, in the presence or absence of 200 pM rhBMP7 (kind gift from Dr. J. McCartney, Curis, Cambridge, MA). The concentrations of TGF-β were selected to be within the physiological range that has been found in serum (9, 11) but less than 1 nM, which usually induces maximal effects in most cell types. In individual experiments, we examined the effects of TGF-β and BMP7 on collagen type IV (col IV), fi-
bronectin (FN), and thrombospondin (TSP), on connective tissue growth factor (CTGF) and matrix metalloproteases (MMP) and their activity regulator plasminogen activator inhibitor (PAI-1).

Effects of BMP7 on collagen α1IV and FN mRNA levels. Near-confluent mesangial cells in six-well plates were grown arrested by serum starvation in medium containing 0.1% BSA. Cells were then incubated with 200 pM rhBMP7, 200 pM rhTGF-β1, or both for 72 h (n = 6 each). Total RNA was extracted with RNA-Stat 60 reagent and the procedure recommended by the manufacturer (Tel Test, Friendswood, TX). mRNA levels encoding FN or collagen α1IV (col4A1) were measured by quantitative reverse-transcription polymerase chain reaction. Random-primed first strand cDNA was synthesized from RNA with Omniscript Reverse Transcriptase and random primers (Qiagen, Valencia, CA). Aliquots of cDNA were amplified with Taq polymerase (Qiagen) and the following species- and gene-specific primers. Col4A1: 5'-TAGGTGTCAGCAATTAGG-3' (sense) and 5'-TCACCTCAGCATAGTGTCGCCG-3' (antisense); FN: 5'-ATGCCAGATTGTCACACACG-3' (sense) and 5'- TGCCGCAACTCTGCTGCGCGGCG-3' (antisense). The optimal number of cycles for amplification in the linear range was determined in pilot assays. For col4A1, 42 cycles of 94°C for 45 s, 60°C for 45 s, and 72°C for 60 s; and for FN, 30 cycles of 94°C for 45 s, 58°C for 45 s, and 72°C for 60 s were found optimal followed by 72°C for 7 min to complete the last cycle. 18S RNA was used as endogenous amplification standard. The optimal ratio of 18S primers to 18S compatimers (Qiagen) for col4A1 was 1:9 and for FN it was 3:7 to derive similar 18S yields compared with that of col4A1 and FN, respectively. PCR products were electrophotoretedly resolved in 2% agarose gels containing ethidium bromide, illuminated with UV light and analyzed by digital densitometry using Alpha DigiDoc 100 (Alpha Innotech, San Leandro, CA).

Effects of BMP7 on TGF-β-induced accumulation of cell-associated FN and col IV. Mesangial cells were incubated without or with rhTGF-β1 (200 pM) in the presence or absence of rhBMP7 (200 pM) for 72 h, n = 6 each. Media were removed and cell layers were washed three times with ice-cold PBS. Cells and matrix were lysed with 2× reducing Laemmli buffer, scraped off the plates, sonicated with three short 1-s bursts at 4 W, and heated at 80°C for 10 min. Proteins were resolved by SDS-PAGE in 5% gels. Resolved proteins were electrotransferred onto nitrocellulose. Membranes were blocked with 5% dry milk (DM) in Tris-buffered saline containing 0.05% Tween 20 (TBBS). For visualization of FN, membranes were incubated with anti-FN monoclonal antibody (1:1,500; BD-Biosciences, Palo Alto, CA) and subsequently conjugated with horseradish peroxidase (HRP) antimouse IgG. Bands were visualized by enhanced chemiluminescence and captured on X-ray film. For col IV Western blot, blocked membranes were incubated with biotin-conjugated goat anti-col IV antibody (1:2,000; Southern Biotechnology, Birmingham, AL). Bands were visualized with Neutravidin-HRP (Southern Biotechnology) and subsequent chemiluminescence.

Cell-associated col IV was also measured quantitatively by ELISA as described previously with some modifications (10). Briefly, cells were washed three times with ice-cold PBS, scraped into 3 M guanidine/0.05 M Tris, pH 7.5, and homogenized. Prestained proteins were suspended in incubation buffer (0.1 M NaHCO3, pH 9.8), sonicated, and cleared by centrifugation. Ninety-six-well plates were coated with 100 µl/well of cleared supernatants, each in triplicate, for 48 h at 4°C. Then 100 µl/well of blocking buffer (0.2% BSA in PBS) were added to each well and incubated for 24 h. Wells were washed three times with washing buffer (PBS containing 0.05% Tween 20) and incubated with biotinylated anti-col IV antibody (1:1,500) in incubation buffer (0.2% BSA in PBS, 0.05% Tween 20). Wells were washed six times and incubated with Neutravidin-Avidin-HRP in incubation buffer at room temperature for 1 h. Wells were washed again and 100 µl/well of 0.2% O-phenylenediamine in reaction buffer (200 mM Tris-HCl, 150 mM NaCl, pH 6.0, 0.01% H2O2) were added to each well. Plates were incubated for 45 min in the dark and 50 µl/well of 1.3% Na sulfite in 4 M H2SO4 were added. Color intensity was measured at 492 nm in an ELISA-plate reader (Molecular Devices, Menlo Park, CA). Results were expressed as percent mean control (cells incubated in the absence of TGF-β and BMP7).

Effect of BMP7 on TGF-β-induced soluble col IV and TSP. Near-confluent mesangial cells were incubated with protein-free medium containing TGF-β (0, 50, 100, or 200 pM) in the presence or absence of BMP7 (200 pM), n = 6 each in six-well culture plates for 72 h. Conditioned media were then cleared centrifugation and concentrated 10-fold with spin concentrators (Amicon, Bedford, MA). Col IV was examined by Western blot analysis, aliquots of concentrated conditioned media were electrophoresed in 5% SDS-PAGE minigels and transferred to nitrocellulose. Membranes were blocked and incubated with biotinylated anti-col IV (Southern Biotechnology) and then with Neutravidin-Avidin-HRP (Southern Biotechnology). Bands were visualized with enhanced chemiluminescence.

Soluble col IV was also measured by ELISA in concentrated, conditioned media essentially as described above with the exception that wells were coated with 25 µl of concentrated media in coating buffer.

TSP in conditioned media was examined by Western blotting. Heparin-binding proteins were precipitated from cleared media with 40 µl/ml of a 1:1 slurry of preswollen heparin-conjugated Sepharose CL-6B in PBS (Pharmacia, Piscataway, NJ) and rocking for 4 h at 4°C. Precipitates were washed three times with ice-cold PBS, taken up in reducing Laemmli buffer, and electrophoresed in 7.5% SDS-PAGE gels. After electroblotting of resolved proteins, membranes were blocked with 5% DM/TBBS and immunoblotted with anti-TSP antibody (Santa Cruz Biotechnology, Santa Cruz, CA) and subsequently HRP-anti goat-IgG. Bands were visualized with chemiluminescence.

Effects of BMP7 on TGF-β-induced CTGF. CTGF is thought to mediate some of the fibrogenic actions of TGF-β in some cell types including mesangial cells (6). Thus we tested whether coinubation of mesangial cells with TGF-β in the presence of BMP7 would reduce secreted CTGF levels. Arrested mesangial cells in six-well plates were incubated with rhTGF-β1 (200 pM), rhBMP7 (200 pM), both, or neither (control) for 48 h. Conditioned media were cleared by centrifugation. Heparin-binding proteins were precipitated as described above. Washed precipitates were taken up in nonreducing sample buffer and boiled for 3 min. Proteins were separated in 15% SDS-PAGE gels and electroblotted onto nitrocellulose. Membranes were blocked and incubated with chicken anti-CTGF-IgY (0.5 µg/ml; gift from Fibrogen, South San Francisco, CA) and HRP-anti-chicken IgY. Bands were visualized with chemiluminescence and captured on X-ray film.

MMP activity in mesangial cell-conditioned media. To examine whether BMP7 antagonizes the TGF-β-induced decrease in MMP activity that contributes to the accumulation of ECM, mesangial cells were incubated in media containing TGF-β (0–200 pM) or BMP7 (0 or 200 pM) for 24 h. MMP activity was examined by gelatin zymography in media that
had been cleared by centrifugation. Briefly, 10% SDS-PAGE minigels were prepared with 1 mg/ml presolved gelatin as substrate. Samples in reducing agent-free sample buffer were separated by electrophoresis and fixed in 2.5% Triton X-100 and then developed in 50 mM Tris, 200 mM NaCl, 5 mM CaCl2, 0.02% Brij-35, pH 7.5 for 24 h at 37°C. Gels were stained with 0.5% Coomassie blue R-250 and lightly destained. Additional gels were transferred, and blotted as above, except that polyclonal reducing sample buffer, boiled for 4 min, electrophoresed, was used. Bands were visualized with enhanced chemiluminescence and captured on X-ray film.

Effect of BMP7 on TGF-β-dependent PAI-1 promoter activation. MMPs can be activated by proteolysis of Pro-MMPs by plasmin. This enzyme, in turn, is derived from plasminogen by a proteolysis step requiring urokinase-type plasminogen activator (uPA). The activity of uPA and, hence, MMP2 is negatively regulated by the PAI-1. The PAI-1 promoter contains a TGF-β-regulated response element and TGF-β upregulates PAI-1 transcription (25). We hypothesized that BMP7 may function by antagonizing TGF-β-dependent activation of MMPs by reducing PAI-1 promoter activation by TGF-β. This question was examined in mink lung epithelial cells (MLECs) that had been stably transfected with a construct containing the PAI-1 promoter and luciferase reporter (1) (kindly provided by D. Rifkin, NYU). Cells were grown to confluence in DMEM/F-12 containing G418 and 10% FCS. Cells were washed three times with PBS and then incubated for 16 h with serum/G418-free medium containing 0.1% BSA and TGF-β (0, 50, 100, or 200 pM) in the presence or absence of BMP7 (200 pM), n = 6–8 each. Additional incubations were made with excess 29 nM BMP7. Cells were washed three times with ice-cold PBS and lysed in 20 µl/well of lysis buffer (25 mM Tris-phosphate, pH 7.8, 2 mM DTT, 2 mM 1,2-diaminocyclohexane-N,N,N’,N’-tetraacetic acid, 10% glycerol, 1% Triton X-100). Luciferase activity in cell lysates was measured in a luminometer with a commercially available luciferase reagent using the manufacturer’s instructions (Promega, Madison, WI).

Effects of TGF-β and BMP7 on PAI-1 and MMP2 protein levels. To examine protein levels of secreted PAI-1 and MMP2 in mesangial cells, mesangial cells were incubated with serum/BSA-free medium for 48 h without (control) or with TGF-β or BMP7, each at 200 pM, or both (n = 4 each). Media were cleared by centrifugation. For PAI-1 Western blot assay, aliquots of cleared media were concentrated in spin concentrators (Amicon), taken up in 1× reducing sample buffer, boiled for 5 min, electrophoresed in 10% SDS-PAGE gels, and transferred to nitrocellulose. Blocked membranes were incubated with polyclonal anti-PAI-1 antibody (1:800, Santa Cruz Biotechnology) in TTBS/4% BSA overnight at 4°C, washed, and then incubated with HRP-conjugated second antibody (1:12,000 in TTBS/5% BSA) for 1 h. Bands were visualized with enhanced chemiluminescence and captured on X-ray film.

For MMP2 Western blot analysis, heparin-binding proteins were precipitated from aliquots of cleared media as described above. Washed precipitates were taken up in 1× reducing sample buffer, boiled for 4 min, electrophoresed, transferred, and blotted as above, except that polyclonal anti-MMP2 primary antibody was used at 1:500 (Santa Cruz Biotechnology). For derivation of semiquantitative results, band intensity was measured densitometrically and data were expressed as percentage of mean control.

Statistical analysis. Results are expressed as means ± SE. Significance of differences was examined by analysis of variance and subsequent Newman-Keuls multicomparison test. P < 0.05 is considered to reflect significance of difference.

**RESULTS**

**BMP7 reduces TGF-β-induced accumulation of cell-associated FN and col IV.** At the concentrations tested in the present studies, TGF-β did not significantly raise steady-state FN or col α1IV mRNA levels in cultured murine mesangial cells. Both were also unaffected by BMP7 (Fig. 1). Despite the lack of significantly increased mRNAs, both FN and col IV proteins accumulated during incubation with TGF-β. Cell-associated col IV moderately increased with each of the three TGF-β levels by up to 2.2-fold (Fig. 2). BMP7 did not reduce baseline levels of col IV but ameliorated the TGF-β-induced increase in cell-associated col IV by ~25%.

Cell-associated matrix FN was also increased by TGF-β, almost 2.4-fold compared with control. Coincubation with BMP7 also reduced the rise in FN by almost one-half (Fig. 3).

**BMP7 reduces TGF-β-induced soluble col IV and secreted TSP.** Collagens are secreted by cells and assemble into complex matrix structures that become cell associated. Some of the secreted collagen in cell cultures is present in soluble forms. As shown previously by other investigators, TGF-β raises col IV in mesangial cell media. The soluble col IV that was immunodetected in concentrated, conditioned media increased more than threefold with the highest TGF-β concentration and increased significantly by ~50%, even with the lowest TGF-β concentration (Fig. 4). Coincubation with BMP7 reduced the levels of col IV in media by ~30–40% (Fig. 4). The rise in col IV that is induced by the lowest concentration of TGF-β (50 pM) is actually quantitatively inhibited by BMP7. The rise in col IV that is associated with the two greater levels of TGF-β was significantly reduced but not completely prevented by BMP7 (Fig. 4). Incubation of cells with BMP7 (200 pM) in the absence of TGF-β does not significantly reduce secreted or cell-associated col IV levels.

TSP is also a secreted ECM protein and is known to be induced by TGF-β, in part, through TGF-β-induced...
CTGF (19, 27, 28). As shown in Fig. 5, TGF-β (200 pM) increases the levels of TSP in conditioned media, and this effect of the cytokine is also ameliorated by coinubation with BMP7 at equimolar concentrations. BMP7 reduces TGF-β-induced, secreted CTGF. CTGF is a secreted mediator of many (but not all) effects of TGF-β in fibroblasts and also in mesangial cells (6, 15, 19). Consistent with previous findings from other laboratories, TGF-β increases the levels of CTGF in mesangial cell media in the present studies about threefold (Fig. 6). The accumulation of CTGF in conditioned media was significantly, although not completely, reduced by coincubation with BMP7 at equimolar concentrations (Fig. 6).

BMP7 antagonizes the reduction in MMP2 activity by TGF-β. TGF-β is known to reduce the activity of MMPs in various cell types. Mesangial cells primarily secrete MMP2, and its activity and perhaps levels are downregulated by TGF-β (2, 23, 24). To test whether BMP7 alters the reduced activation that occurs with TGF-β, mesangial cells were incubated with both proteins and zymography was performed on conditioned media. Zymograms with gelatin as substrate showed a single band at 70 kDa, known to represent MMP2 (gelatinase A) (Fig. 7). β-Casein zymography did not visualize any band (not shown). Incubation of cells with TGF-β, 50 and 100 pM, tended to reduce MMP2 activity moderately (P = not significant), but at 200 pM, TGF-β substantially reduced MMP2 activity (P < 0.05; Fig. 7).

BMP7, 200 pM, almost quantitatively prevented the reduction in MMP2 activity that was induced by the two lower concentrations of TGF-β and maintained MMP2 activity at about three-quarters of control even in the presence of TGF-β, 200 pM (Fig. 7).
**BMP7 blocks TGF-β-induced PAI-1 promoter activation.** A major mechanism through which TGF-β down-regulates MMP activities has been demonstrated previously, namely, through increasing PAI-1 levels that blocks upstream steps required for MMP activation. Specifically, PAI-1 reduces the generation of active plasminogen activator (PA) from inactive pro-PA. TGF-β upregulates PAI-1 transcription through smad3 and a smad response element in the PAI-1 promoter. We tested the question of whether BMP7 blocks this action of TGF-β in MLEC s stably expressing a PAI-1 promoter/luciferase reporter construct. As expected, TGF-β substantially increases PAI-1 promoter activity (Fig. 8). Coincubation of the cells with TGF-β in the presence of BMP7, each at 200 pM, reduced PAI-1 promoter activation by ~65% compared with incubation with TGF-β alone (Fig. 8). At excessively high levels, 29 nM, BMP7 downregulates PAI-1 promoter activity by ~50% below control levels even in the absence of TGF-β (not shown).

**BMP7 reduces TGF-β-induced PAI-1 levels in mesangial cell media.** Consistent with the findings in MLEC s, incubation of mesangial cells with TGF-β increases accumulation of PAI-1 protein in media (Fig. 9). This rise in accumulated PAI-1 was substantially less in media from cells that were coincubated with TGF-β in the presence of BMP7. These findings indi-
cate that BMP7 also blocks TGF-β-induced PAI-1 in mesangial cells, presumably also by antagonizing the increase in PAI-1 transcription.

**BMP7 blocks TGF-β-induced decrease in MMP2 levels in mesangial cells.** Incubation of mesangial cells with TGF-β also substantially reduces the levels of MMP2 that were recovered from media by precipitation with heparin-Sepharose (Fig. 9). The reduction in secreted MMP2 was prevented, in part, by coinubation with BMP7. In the MMP2 Western blot, only a single band corresponding to ~70 kDa apparent molecular weight band in the zymograms but not higher molecular weight forms was found. This may suggest that perhaps only free but not complexed MMP2 was detected.

**DISCUSSION**

Glomerular sclerosis and renal interstitial fibrosis are features of most progressive renal diseases and cause renal failure. TGF-β has been recognized as an important profibrogenic agent in the kidney, and its antagonism such as with administration of neutralizing antibodies or soluble receptor decoy proteins ameliorates nephron fibrosis in experimental glomerular and renal diseases (14, 32). TGF-β contributes to progressive glomerular and interstitial fibrogenesis by increasing gene expression of some ECM proteins, but a major action of this cytokine is the reduction of degradation and thereby increasing half-lives and, hence, progressive extracellular deposition of matrix proteins (2, 26, 30). To this end, in mesangial cells, TGF-β reduces MMP2 activation but also its levels as shown in the present studies and in previous experiments by other investigators (2). MMP2 contributes to the proteolytic degradation of several different matrix proteins (including collagens, elastin, FN, and laminin) and contributes to the proteolytic activation of several other MMPs (5). Downregulation of MMP2 activity, therefore, contributes to accumulation of several different ECM proteins. Moreover, maintenance of MMP2 activity by BMP7 may have important antifibrogenic effects.

BMP7 is another member of the TGF-β superfamily of cysteine-knot growth factors. It plays major roles during renal and eye development (8). In adults, BMP7 expression is very limited, and tissues with greatest levels include the kidney (7, 22). Its function in the kidney is largely unknown. During progression of chronic renal diseases such as diabetic nephropathy and obstructive nephropathy, renal BMP7 levels decrease substantially, which may be caused, in part, by rising TGF-β (12, 29).

Exogenous administration of rhBMP7 to rats with experimental obstructive nephropathy, streptozotocin-induced diabetic nephropathy, or other experimental renal diseases ameliorates the development of glomerular sclerosis (7, 12, 13, 16, 18, 20). Fibrosis in these models is, at least in part, TGF-β dependent, which gives rise to the possibility that BMP7 antagonizes profibrogenic actions of TGF-β in the kidney.

The present experimental studies examine this hypothesis. Mesangial cells are the pivotal cell type in the glomerulus elaborating the ECM proteins that accumulate to progressive glomerular fibrosis in many glomerular diseases, and FN and col IV contribute to glomerular scar formation. TGF-β-dependent accumulation of these ECM proteins results from an imbalance between increased production and/or reduced metabolism of individual ECM proteins. Although the present studies use an in vitro model of SV40-transfected murine mesangial cells, previous observations from other
laboratories strongly suggest that the findings apply to the pathogenesis of glomerular fibrosis in vivo (21, 26).

The present in vitro experiments in cultured mesangial cells show that the TGF-β-induced accumulation of FN, col IV, and TSP can be reduced by coincubation with BMP7, suggesting an antifibrogenic role of the latter protein. A major although perhaps not exclusive mode of action of BMP7 is to block the TGF-β-induced downregulation of MMP2, i.e., to prevent TGF-β’s reduction of ECM protein metabolism. MMP activity is regulated by activators (uPA) that upregulate urokinase activity, which, in turn, activates MMPs, and by tissue inhibitors of metalloproteases that block MMPs. PAI-1 is a principal antagonist to the activity of uPA, and the upregulation of its promoter activity by TGF-β is a major mechanism through which TGF-β downregulates glomerular ECM degradation (26). Increased transcription of the PAI-1 gene by TGF-β is mediated by direct interaction of TGF-β-activated smad3/4 complex with a smad response element in the PAI-1 promoter (25). In the present studies, we tested whether BMP7 inhibits this particular step in the profibrogenic chain of actions of TGF-β. Indeed, BMP7 ameliorates the activation of PAI-1 transcription that normally occurs on incubation with TGF-β. This likely causes or importantly contributes to the maintenance of nearnormal MMP activity despite the presence of TGF-β. This cytokine has previously been shown to also downregulate MMP2 levels in mesangial cells (24). Findings in the present studies confirm a reduction of (free) MMP2 in media from TGF-β-conditioned cells (Fig. 9). Thus TGF-β may reduce both levels and PAI-1-driven activation of MMP2, and both effects are apparently blocked by BMP7.

In addition to its effects on PAI-1 and MMP2, BMP7 also antagonizes secretion of TSP and CTGF that is induced by TGF-β. The moderate reduction in CTGF may also mediate some of the antifibrogenic effects of BMP7. This may include TSP, which is, in part, regulated through CTGF (27). Detailed mechanisms of how BMP7 may regulate CTGF are not revealed by the present studies.

In summary, the present experimental studies indicate that BMP7 partially blocks TGF-β-induced CTGF as well as accumulation of col IV, FN, and TSP in cultured mesangial cells. BMP7 antagonizes TGF-β-induced downregulation of MMP2 and increased transcription and secretion of PAI-1, suggesting that BMP7 opposes downregulation of matrix degradation by TGF-β. Thus BMP7 blocks several profibrogenic activities of TGF-β in mesangial cells.

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