Histol Histopathol (2003) 18: 217-224

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

Review

Exploring the connection between chronic renal fibrosis and bone morphogenic protein-7

R. Kalluri and M. Zeisberg

Program in Matrix Biology, Department of Medicine, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, USA

Summary. Tubulointerstitial fibrosis is a hallmark feature of chronic renal injury. Specific therapies to control the progression of renal fibrosis towards end-stage renal failure are still limited. Transforming growth factor-ß1 (TGF-ß1) has been identified as a major mediator of renal fibrosis. Recent reports have suggested that Bone Morphogenic Protein-7 (BMP-7), another member of the TGF-ß superfamily, accelerates repair of acute renal injury and ameliorates progression of chronic renal fibrosis in a variety of animal models. Interestingly, BMP-7, an endogenous molecule which is present in the normal kidney, vastly decreases its expression during renal injury. Although, the mechanism of BMP-7 action in the kidney is not yet fully understood, the idea of an endogenous molecule with reno-protective function is intriguing.

Key words: Bone Morphogenic Protein-7 (BMP-7), Osteogenic Protein-1 (OP-1), Transforming Growth Factor-ß1 (TGF-ß1), Renal Fibrosis, Tubular Epithelial Cells (TECs)

Overview

Chronic renal fibrosis represents about an 18 billion dollar expense annually for the health care system in the United States of America and Europe. Recent studies have suggested that an endogenous molecule called BMP-7, might be a potent inhibitor of progressive renal injury and fibrosis in mice. Research on this molecule with respect to renal injury began, when Creative Biomolecules, Inc./ Curis, Inc. made an initial observation that BMP-7 might have a therapeutic activity in the injured kidney. In a visionary move, this company solicited several researchers around the United States and Europe during the period between 1997 and 1999 to explore connection between BMP-7 and the injured adult kidney. Our laboratory, along with a few others, were picked for this task. BMP-7 is an approved drug for the healing of fractures in both the United States and Europe. This review will highlight existing information about BMP-7 and its potential role in the kidney during health and disease.

Offprint requests to: Dr. Raghu Kalluri, Associate Professor of Medicine, Harvard Medical School, Program in Matrix Biology, Department of Medicine, Dana 514, Beth Israel Deaconess Medical Center, 330 Brookline Avenue. Boston, MA 02215, USA. Fax: 617-667-2562. e-mail: rkalluri@caregroup.harvard.edu

Chronic renal fibrosis

The progression of chronic renal disease and fibrosis still represents one of the biggest challenges in nephrology, as it leads a large number of patients towards end stage renal failure (ESRF), thus requiring long term dialysis replacement therapy (Pastan and Bailey, 1998; Remuzzi and Bertani, 1998). Although establishment of dialysis and organ transplantation have been sufficient to circumvent the instant fatality due to ESRF (Pastan and Bailey, 1998), both options still present significant problems, such as high costs, limited availability, reduced quality of life and overall prognosis (Bruns et al., 1998; Pastan and Bailey, 1998). Despite recent progress in understanding the pathogenesis associated with chronic progressive renal disease and fibrosis, current paradigms for treatment of chronic renal failure are still aimed at delaying the onset of ESRF rather than prevention or reversal (Hebert et al., 2001).

The most common diseases which lead to ESRF differ significantly in their underlying primary patho-mechanisms (Nath, 1992; Anderson, 1996; Eddy, 1996). Glomerulonephritis due to primary glomerular inflammation, metabolic diseases such as diabetes mellitus, cystic nephropathies such as polycystic kidney disease, interstitial nephritis due to primary interstitial inflammation, and primary vasculopathies are among the leading causes of ESRF (Anderson, 1996; Fogo, 2000) (Becker and Hewitson, 2000; Muller et al., 2000). Despite the diversity of primary patho-mechanisms associated with these different kidney diseases, they all lead to an indistinguishable scarred/fibrotic kidney (Strutz and Muller, 1995; Fogo, 2000). The observation

that chronic renal failure seems to progress independent of the underlying disease, has resulted in a speculation for the existence of a common pathogenic pathway leading to fibrosis and eventual end stage renal disease (ESRD) (Brenner et al., 1982; Bohle et al., 1989; Remuzzi and Bertani, 1998).

Common pathogenic pathways leading to ESRD are characterized by interstitial fibrosis and tubular atrophy (Bohle et al., 1979; Fogo, 2000; Muller et al., 2000). Tubulointerstitial fibrosis is the single most important pathological event that correlates with renal dysfunction and the final fate of the kidney (Bohle et al., 1979; Eddy, 1996; Remuzzi and Bertani, 1998). Fibrosis is defined as an excessive matrix-deposition which leads to destruction of parenchymal tissue structure, leading to impairment of organ function (Mutsaers et al., 1997). The development of renal fibrosis seems to follow a similar pathway as that of normal wound healing (Nodder and Martin, 1997; Strutz and Muller, 1999). However, unlike wound healing where the repair process is self-contained and undergoes programmed resolution (Strutz and Muller, 1999), renal fibrosis is associated with chronic progressive scar tissue formation, which results in excessive deposition of extracellular matrix (ECM) without resolution (Eddy, 1996; Strutz and Muller, 1999). Factors that initiate and activate renal fibrosis and wound healing seem significantly similar, hence, it can be envisioned that renal fibrosis is nothing but wound healing with a loss of programmed capacity for resolution.

Pathomechanisms of renal fibrosis

It is suggested that chronic fibrogenesis is determined by three factors: 1) a continuous primary insult or stimulus, making the fibrotic process a long term event, 2) recruitment of inflammatory cells, associated with activation of resident cells, 3) excessive synthesis of collagen and other ECM components. Renal fibrogenesis is initiated by an insult that leads to an inflammatory reaction with an influx of mononuclear cells into the interstitium (Strutz and Muller, 1995). The infiltrating cells release profibrogenic growth factors that stimulate local fibroblasts and tubular epithelial cells (Becker and Hewitson, 2000; Zeisberg et al., 2001). In the second phase of fibrogenesis, known as the inflammatory matrix synthesis phase, fibroblasts predominantly synthesize ECM products (Strutz and Muller, 1995). This stage of disease is still dependent on the mononuclear infiltrate and considered potentially reversible (Eddy, 1996). The third phase, known as the phase of post-inflammatory matrix synthesis, is characterized by an ongoing matrix synthesis despite possible resolution or attenuation of the primary inflammatory stimulus (Muller et al., 2000; Strutz et al., 2000; Zeisberg et al., 2000). Unlike normal wound repair, a significant number of proliferating fibroblasts remain detectable in the scarred tissue (Strutz et al., 2000; Zeisberg et al., 2000). Thus, the scar formation process remains activated, instead of being terminated like in a normal wound healing setting (Strutz et al., 2000). Removal of deposited matrix is considered impossible at this stage of disease (Strutz and Muller, 1995; Eddy, 1996).

The role of tubular epithelial cells in renal fibrosis

The main cellular events facilitating tubulointerstitial fibrosis are the activation of fibroblasts and tubular epithelial cells (Zeisberg et al., 2001). Tubular epithelial cells are increasingly being recognized as major contributors to fibrogenesis (Becker and Hewitson, 2000; Suzuki et al., 2001). They mediate initiation of interstitial inflammation by release of chemokines, pro-fibrotic growth factors and serve to regulate immune infiltrates via upregulation/expression of adhesion molecules (Schlondorff et al., 1997; Abbate et al., 2002; Segerer et al., 2000; Shappell et al., 2000). Upon initiation of tubulointerstitial disease, tubular epithelial cells are thought to undergo a cascade of cellular events that lead to tubular atrophy (Becker and Hewitson, 2000; Zeisberg et al., 2001). Activated tubular epithelial cells undergo hypertrophy due to G1 phase arrest (Shankland and Wolf, 2000). In later phases of fibrogenesis, tubular epithelial cells may undergo epithelial to mesenchymal transition (EMT) and/or apoptosis, both contributing to tubular atrophy (Strutz et al., 1996; Becker and Hewitson, 2000; Bergin et al., 2000). Several stimuli, such as high glucose, hypoxia or the disruption of basement membranes (BM), have been identified to initiate EMT of renal tubular cells (Orphanides et al., 1997; Strutz and Muller, 2000; Park et al., 2001; Zeisberg et al., 2001).

The growth factor, TGF-ß1, has been identified as a mediator of hypertrophy (Shankland and Wolf, 2000), EMT (Okada et al., 1997; Fan et al., 1999) and apoptosis (Nowak and Schnellmann, 1996) in tubular epithelial cells, and therefore is currently regarded as the predominant pro-fibrogenic stimulus for tubular cells (Border and Noble, 1994; Sharma et al., 2000). EMT is increasingly being recognized as an important cellular event contributing to tubular atrophy and interstitial fibroblasts (Strutz et al., 1996; Remuzzi and Bertani, 1998; Okada et al., 2000). During EMT, tubular epithelial cells (TECs) activated, lose contact with neighboring cells and tubular BM, acquire typical phenotypic characteristics of fibroblasts and invade the interstitium (Hay and Zuk, 1995; Strutz and Muller, 2000; Yang and Liu, 2001) Strutz et al., 2002; Yang and Liu, 2001; Zeisberg et al., 2002).

Interstitial fibroblasts in renal fibrosis

Due to their capability to secrete most of the ECM associated molecules, fibroblasts are generally considered to play a pivotal role in mediating fibrogenesis (Remuzzi and Bertani, 1998; Zeisberg et al., 2000). In the context of several conditions, such as

inflammation, wound healing and fibrosis, a myofibroblast phenotype (activated fibroblast) appears which resembles vascular smooth muscle cells by morphological and functional parameters (Sappino et al., 1990; Muller et al., 1995). This myofibroblast population is usually α-smooth-muscle actin (α-SMA) positive (Kaissling et al., 1996). Functional characteristics of fibroblast activation typically include increased proliferative activity, increased production of ECM components such as type I collagen and fibronectin, and increased migratory activity (Muller and Rodemann, 1991; Rodemann and Muller, 1991; Eddy, 1996). α-SMA positive fibroblasts appear in acute renal injury as well as during chronic renal damage (Alpers et al., 1994). Factors that determine 'this point of no return' during fibroblast activation are still unclear (Strutz, 1995).

Bone morphogenic protein-7 (BMP-7)

Bone morphogenic proteins, as the name suggests, are a class of protein first identified for their bone-inducive capacity (Kingsley, 1994; Hogan, 1996; Wozney, 1998; Ducy and Karsenty, 2000). In 1965 Urist reported the capacity of demineralized bone to induce ectopic bone formation, suggesting the existence of bone morphogenic proteins (Urist, 1965). BMP-7 was originally purified from bone-inducing extracts of bovine diaphysal bone as an active component (Ozkaynak et al., 1990; Sampath et al., 1990).

BMPs are now known to include a large family of proteins within the TGF-ß superfamily of growth and differentiation factors (Kingsley, 1994; Hogan, 1996; Wozney, 1998). BMP-7 was found to be expressed robustly in the kidneys of mice and rats and this observation began the quest to understand its role in the kidney (Ozkaynak et al., 1991).

BMP-7 is abundantly expressed in the adult human kidney (Ozkaynak et al., 1991; Vukicevic et al., 1998). During kidney development, the formation of glomeruli and tubuli is initiated by condensation of metanephric mesenchyme and consequent mesenchymal epithelial transition (MET) (Qiao et al., 1995; Sorokin and Ekblom, 1992). A number of growth factors and transcription factors have been identified as regulators of this process, which is commonly referred to as "induction of mesenchyme" (Schedl and Hastie, 2000). MET is an important aspect of most developing organs and also might be a significant component of the wound healing process (Ekblom, 1989; Bard and Ross, 1991; Birchmeier and Birchmeier, 1993; Ekblom, 1989; Ouyang, 1998). BMP-7 is speculated to be important for potential induction of MET in the developing kidney (Dudley et al., 1999; Simon et al., 1999; Vukicevic et al., 1994, 1996). Two different groups have independently generated BMP-7-deficient mice and both reported similar phenotypes with minor differences. (Dudley et al., 1995; Luo et al., 1995). Homozygous mutants have microphtalmia, pre-axial polydactyly, severly dysplastic kidneys and they die shortly after birth from renal failure. In these mice, interactions between the epithelial ureter bud and the metanphric mesenchyme are initiated, but branching of the epithelium halts after formation of the S-shaped tubules (Dudley et al., 1995; Luo et al., 1995).

In the normal adult kidney tissue, BMP-7 is detectable in tubular epithelial cells and podocytes (Kopp, 2000). Expression of BMP-7 is most abundant in medullary tubular epithelial cells and in collecting ducts, although expression is also detectable in proximal tubular cells, cortical interstitial cells and podocytes (Vukicevic et al., 1998; Simon et al., 1999). Immunohistochemical studies in adult rat kidneys revealed the presence of BMP-7 in tubular cells of all segments, even though staining was most intense in distal convoluted tubules and collecting ducts (Wang et al., 2001). BMP-7 type II receptors are expressed in the cortex as well as in the medulla of rat kidneys. Interestingly, after intravenous injection of ^{125}I-labeled BMP-7, the relative uptake is higher in the cortex than in the medulla (Bosukonda et al., 2000; Kopp, 2000).

BMP-7 (syn. Osteogenic Protein-1/OP-1), as a member of the TGF-ß superfamily, exerts its biological function via distinct receptors (Miyazono et al., 2001). Proteins of the TGF-ß superfamily signal through heterocomplexes of two different types of signaling receptors termed as type I and type II receptor (Massague, 1992; Heldin et al., 1997; Derynck et al., 1998; Wrana, 2000). Ligand binding leads to association of type I and type II receptors (Attisano and Wrana, 2002; Massague, 2000). Within this complex, the type II receptor kinase activates the type I receptor kinase, which phosphorylates Smads serving as signal transducers (Heldin et al., 1997; Derynck et al., 1998; Massague, 2000; Wrana, 2000). Although TGF-ß1 and BMP-7 belong to the TGF superfamily; they use divergent signaling pathways to impart their actions on cells (Miyazono et al., 2001). ALK-5, which functions via Smad2 and Smad3, is a typical type I receptor for TGF-ß1 (Massague, 1998). BMP-7 forms dimeric complexes with ALK-3 and ALK-6, which transmit signals via Smads 1, 5 and 8, also present intracellularly (Heldin et al., 1997; Derynck et al., 1998; Massague, 2000; Wrana, 2000). Smad4 is common to both TGF-ß1 and BMP-7 mediated pathways (Candia et al., 1997). TGF-ß1 is known to induce a cascade of effects in tubular epithelial cells, leading eventually to events, which contribute to tubulointerstitial fibrosis (Zeisberg et al., 2001). The current hypothesis suggests that TGF-ß1 induces cell hypertrophy in the early phase of fibrotic disease, whereas EMT and apoptosis are involved in later stages of fibrotic disease (Becker and Hewitson, 2000; Zeisberg et al., 2001). Recent publications have demonstrated that BMP-7 receptors ALK-3, ALK-6 and BMPR II are present on proximal tubular epithelial cells (Wang et al., 2001; Gould et al., 2002). The main signaling pathway for BMP-7 in renal tubular cells appears to involve Smad5 (Wang et al., 2001). TGF-ß1

decreases expression of BMP-7 receptors and BMP-7 decreases expression of TGF-ß1 (Wang et al., 2001).

BMP-7 and acute renal injury

Two independent groups investigated renal expression of BMP-7 in a rat model of ischemic acute renal failure and reported similar results. (Vukicevic et al., 1998; Simon et al., 1999). BMP-7 mRNA is mainly detected in tubules of the outer medulla and glomeruli, but it is also present in cortical tubular epithelial cells and the cortical interstitium (Simon et al., 1999; Vukicevic et al., 1998). Expression of BMP-7 is significantly decreased 6 hrs following the ischemic insult and expression levels were still significanly lower, four days later (Vukicevic et al., 1998; Simon et al., 1999). The decrease of BMP-7 expression is most significant in the outer medulla, but also present in the cortex (Vukicevic et al., 1998; Simon et al., 1999). Administration of recombinant human BMP-7 (rhBMP-7) protects the rats from ischemia-induced acute injury (Vukicevic et al., 1998). Tubular necrosis is reduced, pro-inflammatory adhesion molecules are decreased, apoptosis is reduced and functional recovery of the ischemic kidneys is enhanced. Interestingly, binding studies using ^{125}I-BMP-7 demonstrates an abundant binding in the kidney cortex (Vukicevic et al., 1998). Decrease of BMP-7 expression is reciprocal to TGF-ß expression, suggesting an important role for BMP-7 in the maintenance of renal homeostasis in acute injury setting (Simon et al., 1999).

BMP-7 and chronic renal injury

Following the observations, which suggested a role for BMP-7 in the maintenance of tubular homeostasis, hBMP-7 was provided by Creative Biomolecule, Inc./ Curis, Inc. to a number of groups to investigate its potential in inhibiting progression of acute and chronic renal disease, in their favorite renal disease models. Evidence for BMP-7 as a potential anti-fibrotic agent was provided by these independent groups in different models of renal disease.

Hruska et al. reported that administration of rhBMP-7 ameliorated tubular damage and interstitial injury observed in rats five days after unilateral ureteral obstruction (UUO) (Hruska et al., 2000). Treatment with BMP-7 is superior to treatment with Enalapril in this study (Hruska et al., 2000). In a similar study, they also report that BMP-7 accelerates de novo recovery of renal function, when the unilateral ureteral obstruction was removed after three days (Morrissey et al., 2002). In contrast, the same research group did not observe amelioration of renal disease in a mouse model of renal mass ablation (Gonzalez et al., 2002). An explanation for this contradictory result was not presented (Gonzalez et al., 2002). Our research group provided further evidence for BMP-7 as an anti-fibrotic agent in a genetic model of lupus nephritis (Bottiglio et al., 2000). Treatment of MRl/MpJ lpr/lpr mice with BMP-7 over a period of four months significantly prevented interstitial fibrosis and tubular atropy in this long-term model of chronic renal injury (Bottiglio et al., 2000). During the same period, Ikeda et al. demonstrated that treatment with BMP-7 was associated with less interstitial fibrosis in a rat model of overload proteinuria after 6 weeks (Ikeda et al., 2000). Interestingly, in this study, expression levels of TGF-ß mRNA was increased despite a trend towards improvement of renal histology and excretory function (Ikeda et al., 2000). In a different study, Nadim et al. provided evidence that treatment with BMP-7 for 10 weeks reduced glomerulosclerosis and tubulointerstitial injury, independent of effects on the systemic blood pressure in a rat model of 5/6 renal mass ablation (Nadim et al., 2000).

Interestingly, Klahr et al. reported that treatment with BMP-7 ameliorated glomerular pathology and tubulointestitial fibrosis in a model of STZ-induced diabetes (Klahr et al., 2002). Treatment with high doses of BMP-7 (300 mg/kg) from week 16 to week 32 was suggested to partially reverse glomerular hypertrophy and decrease proteinuria (Klahr et al., 2002).

Mechanism of BMP-7 in renal protection and regeneration

Protection of tubular integrity by treatment with BMP-7 is a common finding in these studies of acute and chonic renal injury (Vukicevic et al., 1998; Bottiglio et al., 2000; Hruska et al., 2000; Morrissey et al., 2002). Apoptosis of tubular epithelial cells was significantly reduced in rat models of acute ischemic renal injury and UUO (Vukicevic et al., 1998; Hruska et al., 2000). Kher et al. demonstrated that BMP-7 protects S1 proximal tubular epithelial cells from ATP depletion associated apoptosis (Kher and Bacallao, 2000).

Gould et al. demonstrated that treatment of proximal tubular epithelial cells with BMP-7 reduces pro-inflammatory signals, which can serve as downstream signals originating from tubular epithelial cells during renal fibrogenesis (Gould et al., 2002). BMP-7 reduced TNF-α induced increases in mRNA for interleukin-6, interleukin-1ß, interleukin-8 and monocyte chemotactic protein-1 (Gould et al., 2002). This study failed to demonstrate BMP-7 expression in proximal tubular epithelial cells using a BMP-7/lacZ transgenic mouse model, suggesting that major effects of BMP-7 are mediated via tubular epithelial cells, while it has to be provided from other cellular sources (Gould et al., 2002).

In contrast, Wang et al. demonstrate BMP-7 protein expression in proximal tubular epithelial cells, in distal tubular cells and in collecting ducts of rats using immunohistochemistry (Wang et al., 2001).

BMP-7 protein and mRNA are significantly decreased in a rat model of STZ-induced diabetes (Wang et al., 2001). Neutralization of endogenous BMP-7 in proximal tubular epithelial cells induced expression of

pro-fibrotic molecules fibronectin and collagen α1(III), suggesting that loss of endogenous BMP-7 is associated with pro-fibrotic effects (Wang et al., 2001). Gould et al. demonstrate, that TGF-ß1, the major pro-fibrotic growth factor, reduces BMP-7 expression in proximal tubular epithelial cells (Gould et al., 2002).

Summary and speculations

The central question that emerges from these few, yet provocative studies is, what is the role of BMP-7 in the adult kidney? This is yet unanswered. The role of BMP-7 during kidney development is evident from its expression in the condensing mesenchyme (Vukicevic et al., 1996; Simon et al., 1999; Schedl and Hastie, 2000). It has been speculated that BMP-7 might be involved in inducing condensation of mesenchyme to form epithelium via a dynamic process known as MET (Simon et al., 1999; Vukicevic et al., 1994, 1996). Molecules such as PAX-2 and WT-1 have also been identified to co-localize with BMP-7 in the condensing mesenchyme (Schedl and Hastie, 2000).

In this regard, transcription factor Pax-2 is known to play a key role during embryonic development of kidneys (Dressler et al., 1990, 1993; Dressler and Woolf, 1999; Rothenpieler and Dressler, 1993). During kidney development, Pax-2 is expressed in the undifferentiated mesenchyme in response to ureteric bud induction and continues to be expressed in the developing comma and s-shaped bodies (Dressler and Woolf, 1999). Recent studies by Imgrund et al. show that during the repair phase of mouse experimental acute tubular necrosis, Pax-2 expression was upregulated and re-expression was observed in proximal tubular epithelial cells (Imgrund et al., 1999). The authors suggest that during the regeneration process, development paradigms may be recapitulated in order to restore mature kidney function (Imgrund et al., 1999). Pax-2 and BMP-7 co-express in the condensing mesenchyme in developing kidney (Imgrund et al., 1999; Schedl and Hastie, 2000). These observations provide additional support for BMP-7 as an inducer of MET and other molecular cues associated with renal development in the injured adult kidney.

Further support for the speculated role of BMP-7 in the developing kidney provided through genetic experiments. When the BMP-7 gene was deleted in mice, homozygous BMP-7 mutants die at birth because of renal failure. Kidney development appears normal until it ceases at 11 days, suggesting that BMP-7 is needed for the proliferation and differentiation of the metanephric mesenchyme (Dudley et al., 1995; Luo et al., 1995). These studies provide solid genetic evidence that BMP-7 is important for kidney development.

While the role of BMP-7 in the developing kidney is becoming clearer, the role in adult kidney is still unresolved. Podocytes and glomerular parietal epithelial cells of the Bowman's capsule have been shown to express BMP-7 (Vukicevic et al., 1994; Simon et al., 1999; Gould et al., 2002). While expression of BMP-7 in the tubular epithelial cells is thought to be most abundantly present on distal tubules; expression on proximal tubular epithelial cells is controversial (Vukicevic et al., 1998; Simon et al., 1999; Wang et al., 2001; Gould et al., 2002). This needs to be sorted out. There is enough evidence now to suggest that both acute and chronic injury to the kidney lead to significant reduction in the protein and mRNA levels for BMP-7 (Vukicevik et al., 1998; Simon et al., 1999; Hruska et al., 2000; Wang et al., 2001). Such consistent observations have led to a hypothesis that BMP-7 may provide a potential endogenous protection to the kidney glomerulus and interstitium from injury. Hence loss of this molecule could lead to potential damage to the kidney. While such a hypothesis led to research presented in the earlier sections using BMP-7 as a pharmacological agent, the endogenous reno-protective role for BMP-7 is unproven as yet. The future direction of research in the area must be focused on understanding the *de novo* role of BMP-7 in the adult kidney.

Undoubtedly, these few studies with BMP-7 have raised many intriguing possibilities regarding the use of this molecule as an anti-fibrotic agent in the clinic. While such optimistic thinking is warranted, until the mechanism of action for BMP-7 in the adult injured kidney is understood, the case is still wide open.

Acknowledgements. The authors are supported in part by grants DK51711 and DK55001 from the NIH, a research fund from the Beth Israel Deaconess Medical Center for the Program in Matrix Biology and by a grant from the Deutsche Forschungsgemeinschaft DFG ZE5231-1.

References

Abbate M., Zoja C., Rottoli D., Corna D., Tomasoni S. and Remuzzi G. (2002). Proximal tubular cells promote fibrogenesis by TGF-beta1-mediated induction of peritubular myofibroblasts. Kidney Int. 61, 2066-2077.

Alpers C.E., Hudkins K.L., Floege J. and Johnson R.J. (1994). Human renal cortical interstitial cells with some features of smooth muscle cells participate in tubulointerstitial and crescentic glomerular injury. J. Am. Soc. Nephrol. 5, 201-209.

Anderson S. (1996). Mechanisms of injury in progressive renal disease. Exp. Nephrol. 4, 34-40.

Attisano L. and Wrana J.L. (2002). Signal transduction by the TGF-ßeta superfamily. Science 296, 1646-1647.

Bard J.B. and Ross A.S. (1991). LIF, the ES-cell inhibition factor, reversibly blocks nephrogenesis in cultured mouse kidney rudiments. Development 113, 193-198.

Becker G.J. and Hewitson T.D. (2000). The role of tubulointerstitial injury in chronic renal failure. Curr. Opin. Nephrol. Hypertens. 9, 133-138.

Bergin E., Levine J.S., Koh J.S. and Lieberthal W. (2000). Mouse proximal tubular cell-cell adhesion inhibits apoptosis by a cadherin-dependent mechanism. Am. J. Physiol. Renal Physiol. 278, F758-F768.

Birchmeier C. and Birchmeier W. (1993). Molecular aspects of mesenchymal-epithelial interactions. Annu. Rev. Cell Biol. 9, 511-

540.

Bohle A., Christ H., Grund K.E. and Mackensen S. (1979). The role of the interstitium of the renal cortex in renal disease. Contrib. Nephrol. 16, 109-114.

Bohle A., Kressel G., Muller C.A. and Muller G.A. (1989). The pathogenesis of chronic renal failure. Pathol. Res. Pract. 185, 421-440.

Border W.A. and Noble N.A. (1994). Transforming growth factor beta in tissue fibrosis. N. Engl. J. Med. 331, 1286-1292.

Bosukonda D., Shih M. S., Sampath K.T. and Vukicevic S. (2000). Characterization of receptors for osteogenic protein-1/bone morphogenetic protein-7 (OP-1/BMP-7) in rat kidneys. Kidney Int. 58, 1902-1911.

Bottiglio C., Maeshima Y. and Kalluri R. (2000). Osteogenic Protein-1 Reduces glomerulonephritis and tubulointerstitial fibrosis associated with a mouse model for lupus nephritis. J. Am. Soc. Nephrol. 11, 524A.

Brenner B. M., Meyer W. and Hostetter T.H. (1982). Dietary protein intake and the progressive nature of kidney disease: the role of hemodynamically mediated glomerular injury in the pathogenesis of progressive glomerular sclerosis in aging, renal ablation, and intrinsic renal disease. N. Engl. J. Med. 307, 652-659.

Bruns F.J., Seddon P., Saul M. and Zeidel M.L. (1998). The cost of caring for end-stage kidney disease patients: an analysis based on hospital financial transaction records. J. Am. Soc. Nephrol. 9, 884-890.

Candia A.F., Watabe T., Hawley S.H., Onichtchouk D., Zhang Y., Derynck R., Niehrs C. and Cho K.W. (1997). Cellular interpretation of multiple TGF-ßeta signals: intracellular antagonism between activin/BVg1 and BMP-2/4 signaling mediated by Smads. Development 124, 4467-4480.

Derynck R., Zhang Y. and Feng X.H. (1998). Smads: transcriptional activators of TGF-ßeta responses. Cell 95, 737-740.

Dressler G.R., Deutsch U., Chowdhury K., Nornes H.O. and Gruss P. (1990). Pax2, a new murine paired-box-containing gene and its expression in the developing excretory system. Development 109, 787-795.

Dressler G.R., Wilkinson J.E., Rothenpieler U.W., Patterson L.T., Williams-Simons L. and Westphal H. (1993). Deregulation of Pax-2 expression in transgenic mice generates severe kidney abnormalities. Nature 362, 65-67.

Dressler G.R. and Woolf A. (1999). Pax2 in development and renal disease. Int. J. Dev. Biol. 43, 463-468.

Ducy P. and Karsenty G. (2000). The family of bone morphogenetic proteins. Kidney Int. 57, 2207-2214.

Dudley A.T., Godin R.E. and Robertson E.J. (1999). Interaction between FGF and BMP signaling pathways regulates development of metanephric mesenchyme. Genes Dev. 13, 1601-1613.

Dudley A.T., Lyons K.M. and Robertson E.J. (1995). A requirement for bone morphogenetic protein-7 during development of the mammalian kidney and eye. Genes Dev. 9, 2795-2807.

Eddy A.A. (1996). Molecular insights into renal interstitial fibrosis [editorial]. J. Am. Soc. Nephrol. 7, 2495-2508.

Ekblom P. (1989). Developmentally regulated conversion of mesenchyme to epithelium. FASEB J. 3, 2141-2150.

Fan J. M., Ng, Y.Y., Hill P.A., Nikolic-Paterson D.J., Mu W., Atkins R.C. and Lan H.Y. (1999). Transforming growth factor-beta regulates tubular epithelial- myofibroblast transdifferentiation in vitro. Kidney Int. 56, 1455-1467.

Fogo A.B. (2000). Pathology of progressive nephropathies. Curr. Opin. Nephrol. Hypertens. 9, 241-246.

Gonzalez E.A., Lund R.J., Martin K.J., McCartney J.E., Tondravi M.M., Sampath T.K. and Hruska K.A. (2002). Treatment of a murine model of high-turnover renal osteodystrophy by exogenous BMP-7. Kidney Int. 61, 1322-1331.

Gould S.E., Day M., Jones S.S. and Doai H. (2002). BMP-7 regulates chemokine, cytokine, and hemodynamic gene expression in proximal tubule cells. Kidney Int. 61, 51-60.

Hay E.D. and Zuk A. (1995). Transformations between epithelium and mesenchyme: normal, pathological, and experimentally induced. Am. J. Kidney Dis. 26, 678-690.

Hebert L.A., Wilmer W.A., Falkenhain M.E., Ladson-Wofford S.E., Nahman N.S. and Rovin B.H. (2001). Renoprotection: One or many therapies? Kidney Int. 59, 1211-1226.

Heldin C.H., Miyazono K. and ten Dijke P. (1997). TGF-beta signalling from cell membrane to nucleus through SMAD proteins. Nature 390, 465-471.

Hogan B.L. (1996). Bone morphogenetic proteins in development. Curr. Opin. Genet. Dev. 6, 432-438.

Hruska K.A., Guo G., Wozniak M., Martin D., Miller S., Liapis H., Loveday K., Klahr S., Sampath T.K. and Morrissey J. (2000). Osteogenic protein-1 prevents renal fibrogenesis associated with ureteral obstruction. Am. J. Physiol. Renal Physiol. 279, F130-F143.

Ikeda Y., Jung Y., Kim, H., Oda T., Lopez-Guisa, J., Wing, D., Cai, X., McCartney J. and Eddy A. (2000). Effects of BMP-7 on interstitial fibrosis in rats with overload proteinuria. J. Am. Soc. Nephrol. 11, 530A.

Imgrund M., Grone E., Grone H.J., Kretzler M., Holzman L., Schlondorff D. and Rothenpieler U.W. (1999). Re-expression of the developmental gene Pax-2 during experimental acute tubular necrosis in mice 1. Kidney Int. 56, 1423-1431.

Kaissling B., Hegyi I., Loffing J. and Le Hir M. (1996). Morphology of interstitial cells in the healthy kidney. Anat. Embryol. 193, 303-138.

Kher R. and Bacallao R. (2000). Bone Morphogeni Protei-7 (BMP-7) Confers Protection from ATP Depletion by Inhibiting Apoptosis. J. Am. Soc. Nephrol. 11, 591A.

Kingsley D.M. (1994). The TGF-beta superfamily: new members, new receptors, and new genetic tests of function in different organisms. Genes Dev 8, 133-146.

Klahr S., Morrissey J., Hruska K., Wang S. and Chen Q. (2002). New approaches to delay the progression of chronic renal failure. Kidney Int. Suppl, 23-26.

Kopp J.B. (2000). BMP receptors in kidney. Kidney Int 58, 2237-2288.

Luo G., Hofmann C., Bronckers A.L., Sohocki M., Bradley A. and Karsenty G. (1995). BMP-7 is an inducer of nephrogenesis, and is also required for eye development and skeletal patterning. Genes Dev . 9, 2808-2820.

Massague J. (1992). Receptors for the TGF-beta family. Cell 69, 1067-1070.

Massague J. (1998). TGF-beta signal transduction. Annu. Rev. Biochem. 67, 753-791.

Massague, J. (2000). How cells read TGF-beta signals. Nat. Rev. Mol. Cell Biol. 1, 169-178.

Miyazono, K., Kusanagi, K., and Inoue, H. (2001). Divergence and convergence of TGF-ßeta/BMP signaling. J. Cell Physiol. 187, 265-276.

Morrissey J., Hruska K., Guo G., Wang S., Chen Q. and Klahr S. (2002). Bone morphogenetic protein-7 improves renal fibrosis and

accelerates the return of renal function. J. Am. Soc. Nephrol. 13 Suppl 1, S14-21.

Muller G.A and Rodemann H.P. (1991). Characterization of human renal fibroblasts in health and disease: I. Immunophenotyping of cultured tubular epithelial cells and fibroblasts derived from kidneys with histologically proven interstitial fibrosis. Am. J. Kidney Dis. 17, 680-683.

Muller G.A., Frank J., Rodemann H.P. and Engler-Blum G. (1995). Human renal fibroblast cell lines (tFKIF and tNKF) are new tools to investigate pathophysiologic mechanisms of renal interstitial fibrosis. Exp. Nephrol. 3, 127-133.

Muller GA., Zeisberg M. and Strutz F. (2000). The importance of tubulointerstitial damage in progressive renal disease. Nephrol. Dial. Transplant. 15, 76-77.

Mutsaers S.E., Bishop J.E., McGrouther G. and Laurent G.J. (1997). Mechanisms of tissue repair: from wound healing to fibrosis. Int. J. Biochem. Cell Biol. 29, 5-17.

Nadim M., Rennke H., Taal M., Brenner B., Mackenzie H. and Sayegh M. (2000). Bone morphogenic protein-7 (BMP-7) reduces progressive renal injury in 5/6 nephrectomized rats. J. Am. Soc. Nephrol 11, 625A.

Nath K.A. (1992). Tubulointerstitial changes as a major determinant in the progression of renal damage. Am. J. Kidney Dis. 20, 1-17.

Nodder S. and Martin P. (1997). Wound healing in embryos: a review. Anat Embryol (Berl) 195, 215-228.

Nowak G. and Schnellmann R.G. (1996). Autocrine production and TGF-beta 1-mediated effects on metabolism and viability in renal cells. Am. J. Physiol. 271, F689-F-697.

Okada H., Danoff T.M., Kalluri R. and Neilson E.G. (1997). Early role of Fsp1 in epithelial-mesenchymal transformation. Am. J. Physiol. 273, F563-F574.

Okada H., Inoue T., Suzuki H., Strutz F. and Neilson E.G. (2000). Epithelial-mesenchymal transformation of renal tubular epithelial cells in vitro and in vivo. Nephrol Dial Transplant 15, 44-46.

Orphanides C., Fine L. G. and Norman J.T. (1997). Hypoxia stimulates proximal tubular cell matrix production via a TGF-beta1-independent mechanism. Kidney Int. 52, 637-547.

Ouyang P. (1998). An in vitro model to study mesenchymal-epithelial transformation. Biochem. Biophys Res. Commun. 246, 771-776.

Ozkaynak E., Rueger D.C., Drier, E. A., Corbett, C., Ridge, R. J., Sampath, T. K., and Oppermann, H. (1990). OP-1 cDNA encodes an osteogenic protein in the TGF-beta family. EMBO J. 9, 2085-93.

Ozkaynak E., Schnegelsberg P.N. and Oppermann H. (1991). Murine osteogenic protein (OP-1): high levels of mRNA in kidney. Biochem. Biophys. Res. Commun. 179, 116-123.

Park S.H., Choi H.J., Lee J. H., Woo, C. H., Kim, J. H., and Han, H. J. (2001). High glucose inhibits renal proximal tubule cell proliferation and involves PKC, oxidative stress, and TGF-beta 1. Kidney Int. 59, 1695-705.

Pastan S. and Bailey J. (1998). Dialysis therapy. N. Engl. J. Med. 338, 1428-1437.

Qiao J., Cohen D. and Herzlinger D. (1995). The metanephric blastema differentiates into collecting system and nephron epithelia in vitro. Development 121, 3207-3214.

Remuzzi G. and Bertani T. (1998). Pathophysiology of progressive nephropathies. N. Engl. J. Med. 339, 1448-1456.

Rodemann H.P. and Muller G.A. (1991). Characterization of human renal fibroblasts in health and disease: II. In vitro growth, differentiation, and collagen synthesis of fibroblasts from kidneys with interstitial fibrosis. Am. J. Kidney Dis. 17, 684-686.

Rothenpieler U.W. and Dressler G.R. (1993). Pax-2 is required for mesenchyme-to-epithelium conversion during kidney development. Development 119, 711-720.

Sampath T.K., Coughlin J.E., Whetstone R.M., Banach D., Corbett C., Ridge R.J., Ozkaynak E., Oppermann H. and Rueger D.C. (1990). Bovine osteogenic protein is composed of dimers of OP-1 and BMP-2A, two members of the transforming growth factor-beta superfamily. J. Biol. Chem. 265, 13198-13205.

Sappino A.P., Schurch W. and Gabbiani G. (1990). Differentiation repertoire of fibroblastic cells: expression of cytoskeletal proteins as marker of phenotypic modulations. Lab. Invest. 63, 144-161.

Schedl A. and Hastie N.D. (2000). Cross-talk in kidney development. Curr. Opin. Genet. Dev. 10, 543-549.

Schlondorff D., Nelson P.J., Luckow B. and Banas B. (1997). Chemokines and renal disease. Kidney Int. 51, 610-621.

Segerer S., Nelson P.J. and Schlondorff D. (2000). Chemokines, chemokine receptors, and renal disease: from basic science to pathophysiologic and therapeutic studies. J. Am. Soc. Nephrol. 11, 152-76.

Shankland, S. J., and Wolf, G. (2000). Cell cycle regulatory proteins in renal disease: role in hypertrophy, proliferation, and apoptosis. Am. J. Physiol. Renal Physiol. 278, F515-29.

Shappell S.B., Mendoza L.H., Gurpinar T., Smith C.W., Suki W. N. and Truong L.D. (2000). Expression of adhesion molecules in kidney with experimental chronic obstructive uropathy: the pathogenic role of ICAM-1 and VCAM-1. Nephron 85, 156-166.

Sharma R., Khanna A., Sharma M. and Savin V.J. (2000). Transforming growth factor-beta1 increases albumin permeability of isolated rat glomeruli via hydroxyl radicals. Kidney Int. 58, 131-136.

Simon M., Maresh J.G., Harris S. E., Hernandez J.D., Arar M., Olson M.S. and Abboud H E. (1999). Expression of bone morphogenetic protein-7 mRNA in normal and ischemic adult rat kidney. Am. J. Physiol. 276, F382-F-389.

Sorokin L. and Ekblom P. (1992). Development of tubular and glomerular cells of the kidney. Kidney Int. 41, 657-664.

Strutz F. (1995). The fibroblast-a-(trans-) differentiated cell? Nephrol. Dial. Transplant. 10, 1504-1506.

Strutz F. and Muller G.A. (1999). Interstitial pathomechanisms underlying progressive tubulointerstitial damage. Kidney Blood Press. Res. 22, 71-80.

Strutz F. and Muller G.A. (1995). On the progression of chronic renal disease. Nephron 69, 371-379.

Strutz F. and Muller G.A. (2000). Transdifferentiation comes of age. Nephrol. Dial. Transplant. 15, 1729-1731.

Strutz F., Muller G.A and Neilson E.G. (1996). Transdifferentiation: a new angle on renal fibrosis. Exp. Nephrol. 4, 267-270.

Strutz F., Zeisberg M., Hemmerlein B., Sattler B., Hummel K., Becker V. and Muller G.A. (2000). Basic fibroblast growth factor expression is increased in human renal fibrogenesis and may mediate autocrine fibroblast proliferation. Kidney Int. 57, 1521-1538.

Strutz F., Zeisberg M., Ziyadeh F.N., Yang C.Q., Kalluri R., Muller G.A., and Neilson E.G. (2002). Role of basic fibroblast growth factor-2 in epithelial-mesenchymal transformation. Kidney Int .61, 1714-1428.

Suzuki T., Kimura M., Asano M., Fujigaki Y. and Hishida A. (2001). Role of atrophic tubules in development of interstitial fibrosis in microembolism-induced renal failure in rat. Am. J. Pathol. 158, 75-85.

Urist M.R. (1965). Bone: formation by autoinduction. Science 150, 893-

899.

Vukicevic S., Kopp J.B., Luyten F.P. and Sampath T.K. (1996). Induction of nephrogenic mesenchyme by osteogenic protein 1 (bone morphogenetic protein 7). Proc. Natl. Acad. Sci. USA 93, 9021-9026.

Vukicevic S., Basic V., Rogic D., Basic N., Shih M.S., Shepard A., Jin D., Dattatreyamurty B., Jones W., Dorai, H., Ryan S., Griffiths D., Maliakal J., Jelic M., Pastorcic M., Stavljenic A. and Sampath T. K. (1998). Osteogenic protein-1 (bone morphogenetic protein-7) reduces severity of injury after ischemic acute renal failure in rat. J. Clin. Invest. 102, 202-214.

Vukicevic S., Latin V., Chen P., Batorsky R., Reddi A.H. and Sampath T.K. (1994). Localization of osteogenic protein-1 (bone morphogenetic protein-7) during human embryonic development: high affinity binding to basement membranes. Biochem. Biophys. Res. Commun. 198, 693-700.

Wang S.N., Lapage J. and Hirschberg R. (2001). Loss of tubular bone morphogenetic protein-7 in diabetic nephropathy. J. Am. Soc. Nephrol. 12, 2392-2399.

Wozney J.M. (1998). The bone morphogenetic protein family: multifunctional cellular regulators in the embryo and adult. Eur. J. Oral Sci. 106 Suppl 1, 160-166.

Wrana J.L. (2000). Regulation of Smad activity. Cell 100, 189-192.

Yang J. and Liu Y. (2001). Dissection of key events in tubular epithelial to myofibroblast transition and its implications in renal interstitial fibrosis. Am. J. Pathol. 159, 1465-1475.

Zeisberg M., Bonner G., Maeshima Y., Colorado P., Muller G.A., Strutz F. and Kalluri R. (2001). Renal fibrosis: collage composition and assembly regulates epithelial-mesenchymal transdifferentiation. Am. J. Pathol. 159, 1313-1321.

Zeisberg M., Maeshima Y., Mosterman B. and Kalluri R. (2002). Renal fibrosis: extracellular matrix microenvironment regulates migratory behavior of activated tubular epithelial cells. Am. J. Pathol. 160, 2001-2008.

Zeisberg M., Strutz F. and Muller G.A. (2000). Role of fibroblast activation in inducing interstitial fibrosis. J. Nephrol. 13 Suppl 3, S111-S120.

Zeisberg, M., Strutz, F., and Muller, G. A. (2001). Renal fibrosis: an update. Curr. Opin. Nephrol. Hypertens. 10, 315-320.

Accepted August 27, 2002