

Review

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

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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- β 1 (TGF- β 1) 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- β 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- β 1 (TGF- β 1), 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.

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 pathomechanisms (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 pathomechanisms 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- β 1, 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-inductive capacity (Kingsley, 1994; Hogan, 1996; Wozney, 1998; Ducky 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- β 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 microphthalmia, pre-axial polydactyly, severely dysplastic

kidneys and they die shortly after birth from renal failure. In these mice, interactions between the epithelial ureter bud and the metanephric 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- β superfamily, exerts its biological function via distinct receptors (Miyazono et al., 2001). Proteins of the TGF- β 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- β 1 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- β 1 (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- β 1 and BMP-7 mediated pathways (Candia et al., 1997). TGF- β 1 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- β 1 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 BMPRII 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- β 1

decreases expression of BMP-7 receptors and BMP-7 decreases expression of TGF- β 1 (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 significantly 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- β 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 MRI/MpJ lpr/lpr mice with BMP-7 over a period of four months significantly prevented interstitial fibrosis and tubular atrophy 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- β 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 tubulointerstitial 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 chronic 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-1 β , interleukin-8 and monocyte chemoattractant 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 $\alpha 1$ (III), suggesting that loss of endogenous BMP-7 is associated with pro-fibrotic effects (Wang et al., 2001). Gould et al. demonstrate, that TGF- $\beta 1$, 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 (Vukicevic 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.

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