Bone morphogenic protein-7 and the kidney: current concepts and open questions

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Keywords: Bone Morphogenetic Protein-7 (BMP-7); Fibrosis; Epithelial Mesenchymal Transition (EMT); Chronic Kidney Disease (CKD); Chronic Renal Failure (CRF)

Introduction

Several recent studies have demonstrated unequivocally that administration of bone morphogenetic protein-7 (BMP-7) has a therapeutic effect in various animal models of acute and chronic renal injury (Table 1). However, the underlying mechanisms of BMP-7 action in the kidney remained largely unknown in these initial reports. Here, novel aspects regarding the biology of BMP-7 in the kidney will be discussed.

What is BMP-7?

BMP-7, also known as Osteogenic protein-1 (OP-1), is one of 15 currently known BMPs, which are structurally and functionally related and which are part of the transforming growth factor β (TGF-β) superfamily of growth factors [1]. BMP-7 was originally identified as a regulator of cartilage and bone formation [2]. However, BMPs have also been shown to regulate the growth, differentiation, chemotaxis and apoptosis of various cell types, including epithelial, mesenchymal, haematopoietic and neuronal cells [3]. BMPs are highly conserved across animal species and mature human and mouse BMP-7 share 98% amino acid sequence identity [4]. BMP-7 is synthesized as a large precursor protein and the mature, biologically active BMP-7 is generated by proteolytic removal of the signal peptide and pro-peptide [5]. The mature BMP-7 is a glycosylated...
disulphide-linked homodimeric protein consisting of about 36 kDa [5]. BMP-7 is widely expressed in developing tissues, performing functions in various organs [1]. Gene targeting experiments in mice revealed that BMP-7 is indispensable for normal development of the kidney, eye and autopod. In the adult, BMP-7 expression is highest in the kidney, cartilage and bone [6,7].

What is the role of BMP-7 in the developing kidney?

With regard to the kidneys, BMP-7 was first recognized for its relevance during kidney development, as BMP-7-deficient mice have dysplastic kidneys and die shortly after birth [6,7]. During normal kidney development in mice (Figure 1), BMP-7 is first expressed at embryonic day
11 post-coitum in the ureteric duct, and expression in derivates of the ureteric duct is maintained throughout development [8]. Furthermore, the induced mesenchyme (iM), which will give rise to the glomeruli and most parts of the tubular apparatus, expresses BMP-7 in an autocrine manner [9]. In the BMP-7-deficient embryos, branching of the ureteric duct, condensation of the metanephric mesenchyme (MM) and differentiation of epithelial structures is impaired [6]. Even though it can be challenging to delineate specific functions of BMPs due to overlapping specificities, various studies have established that BMP-7 is an important mediator of the morphogenesis of the ureteric bud (UB), that it acts as a survival factor for the stromal cell population adjacent to the nephrogenic mesenchyme, and that it mediates the mesenchymal–epithelial transition (MET) of the iM in concert with various morphogens such as FGF-2 and FGF-8.

How does BMP-7 protect the kidney from injury?

The importance of BMP-7 for the kidney was revealed by several studies that demonstrated unequivocally that administration of recombinant human BMP-7 could protect the kidney in various animal models of acute and chronic renal failure (CRF) (Table 1). All of these studies but one (which evaluated a model of diabetic nephropathy in which tubulointerstitial fibrosis was not observed) suggested that the principal target of BMP-7 in the kidney were tubular epithelial cells (TECs). In recent years, the pivotal role of TECs, the most abundant cell type in the kidney, is becoming increasingly clear [10] during the progression of chronic renal disease. TECs contribute in at least three distinct ways to the progression of tubulointerstitial fibrosis (Figure 1A). TECs are important in the initiation of interstitial inflammation (as they are a major source of chemokines (such as MCP-1), cytokines (such as interleukin-6) and growth factors (such as tumour necrosis factor-α). Furthermore, TECs contribute to the progression of renal fibrosis by undergoing an epithelial–mesenchymal transition (EMT), leading to accumulation of activated fibroblasts in the interstitium [11]. In addition to EMT, apoptosis of TECs is the principal mechanism which leads to loss of viable TECs and tubular atrophy [10]. Recent studies have demonstrated that BMP-7 can directly interfere with pro-fibrogenic functions in TECs: Gould and co-workers [12] demonstrated that BMP-7 decreased secretion of pro-inflammatory cytokines and growth factors by TECs. Similarly, Zhang and colleagues [13] found that BMP-7 interfered with the secretion of TGF-β by TECs. In our studies, we demonstrated that BMP-7 could reverse TGF-β-induced EMT, similar to its role during kidney development [14]. It seems that BMP-7 does not impact apoptosis of TECs, however [15].

How is the biological activity of endogenous BMP-7 in the kidney regulated?

While BMP-7 expression is high in the healthy kidney, expression levels decrease rapidly in animal models of acute renal failure, but return to baseline levels as the kidney regenerates from the initial insult [16,17]. Based on these findings, it has been suggested that it is the BMP-7 expression which determines the biological activity of endogenous BMP-7 in the kidney in health and disease [17]. Consequently, thinking evolved that lack of endogenous BMP-7 expression could be compensated by therapeutic administration of recombinant BMP-7 [17]. However, recent findings suggest that the regulatory mechanisms involving the biological activity of endogenous BMP-7 in the kidney are far more complex: while in the normal kidney BMP-7 expression is high, nuclear staining for phosphorylated Smad1 (which indicates active BMP-7 signaling), is low in proximal tubular epithelial cells (PTEC) [14]. Furthermore, in the setting of chronic renal disease, BMP-7 expression does not appear to correlate with progression of disease as it does in the setting of acute renal injury (it can be decreased, normal or even increased), suggesting that BMP-7 expression is not solely responsible for the biological activity of BMP-7 in the kidney. The biological activity of BMP-7 appears to be controlled at various levels in the kidney (Figure 2). BMP-7 is predominantly expressed in the collecting duct and the distal tubule [12]. It is secreted as a complex consisting of a growth factor homodimer, non-covalently associated with two pre-domain propeptide chains [18]. This complex has a high affinity with certain extracellular matrix constituents and a recent study suggested that the BMP-7 complex is stored bound to fibrillin-1 in the kidney [18]. Little is known about how BMP-7 is mobilized from the ECM and how the pro-domain is cleaved off to generate mature BMP-7. Several molecules have been identified that bind to mature BMP-7, acting as positive or negative regulators of BMP-7 activity in the kidney [3]. The BMP antagonists function through direct association with BMPs, thus prohibiting BMPs from binding to their cognate receptors [3]. Such extracellular inhibitors of BMP signaling include Noggin, Gremlin, CRIM1, DAN/Cerebrus and vertebrate chordin [3]. In this regard, a recent study suggested that USAG-1 (urine sensitization-associated gene-1) functions as a kidney-specific regulator of BMP-7 activity [19]. As opposed to these negative regulators of BMP-7 activity, the recently identified Kielin/Chordin-like protein (KCP) is an extracellular protein that enhances BMP-7 activity by increasing BMP-7 binding to its receptor [20]. While Chordin blocks BMP activity, the structurally related KCP/Crim2 enhances interactions between BMPs and their receptors [20]. At the site of the target cells, signal transduction in the BMP-7 in general is initiated by ligand binding to a receptor complex composed of two type-I receptors and two type-II receptors [21]. Three different BMP type-I receptors (activin receptor-like kinase ALK2,
ALK3 and ALK6) and three BMP type-II receptors (activin type-IIA receptor ActRIIA, activin type-IIB receptor ActRIIB and BMP type-II receptor BMPRII), have been identified [21]. How these receptors are involved in the regulation of BMP-7 in PTEC is not fully understood as yet. Studies which utilized I125-labeled BMP-7 suggested that the BMPRII receptor is constitutively expressed on PTEC, while expression of the type-I receptors may be regulated in the disease setting [22]. Due to lack of reliable reagents, it is not known which of the type-I receptors are responsible for BMP-7 signaling in vivo. However, our studies, which utilized the overexpression of constitutively active Alk3 receptor in PTEC in vitro, suggest an involvement of Alk3 [14]. BMP-7 binding to its receptors induces phosphorylation of the type-I receptor by the type-II receptor, which leads to phosphorylation of cytoplasmatic receptor-activated Smads [23]. BMP-7 signals through Smad1, Smad5 and Smad8, which form heteromeric complexes with Smad4, which in turn translocates from the cytoplasm to the nucleus to regulate gene expression [23].

Is there a role for kidney derived BMP-7 outside the kidney?

In adults, BMP-7 is predominantly expressed in kidney and bone. However, BMP-7 receptors are expressed in various organs. BMP-7 is constantly present in the circulating blood stream at a concentration of 150–300 pg/ml [17]. This suggests the intriguing scenario that BMP-7, which is produced in the kidneys, is constantly released into the circulation, functioning at distant sites in a hormone-like manner. Such thinking has been explored by Davies et al. and Lund et al. [24,25] with regard to bone metabolism associated with renal disease. In two independent studies they could demonstrate that in rat models of osteodystrophy due to renal mass ablation, administration of recombinant BMP-7 could successfully inhibit the bone disorder which occurs in these rats. This suggests that BMP-7, which is required for a normal bone metabolism, stems from the kidney and that the osteodystrophy, which is often observed in patients with CRF, is a direct consequence of decreased BMP-7 release [25].

How can these insights into the biology of BMP-7 impact on clinical nephrology?

Even though several open questions remain to be answered, three different scenarios of a clinical application of BMP-7 in the setting of chronic renal disease deserve consideration: (1) The utility of BMP-7 as a biomarker to monitor or even to predict the progression of chronic renal disease; (2) a use...
for BMP-7 to substitute for the loss of circulating BMP-7 in patients with CRF in order to prevent osteodystrophy and (3) a possible application of recombinant BMP-7 as a therapeutic drug to treat chronic progressive renal disease.

Due to the complex regulation of BMP-7 activity in the kidney it appears that quantification of BMP-7 expression and protein levels has limited utility to serve as a biomarker for CRF. However, the circulating BMP-7 levels deserve further consideration. Studies by Lund and co-workers [25] suggested that decreased BMP-7 levels directly correlate with a loss of viable renal mass. While reliable assays to measure circulating BMP-7 are not available yet, the possibility of measuring circulating BMP-7 as a marker for chronic renal fibrosis should be further explored. Similarly, the consequences of decreased systemic BMP-7 levels deserve further consideration.

The central question though remains, whether BMP-7 could function as a drug to treat chronic renal fibrosis. While current animal studies suggest that systemic BMP-7 administration appears to be safe (in our studies we did not observe ectopic bone formation after 4 months of treatment in mice), the question of efficacy can only be tested in relevant clinical trials. In this regard, novel insights into the complexity of the regulatory mechanisms of BMP-7 activity in the kidney raise the concern that reno-protective BMP-7 pathways cannot be utilized in everyone (see aforesaid) and it can be envisioned that subsets of patients with a favourable receptor status are more likely to respond to treatment with BMP-7. Regulatory BMP-7 pathways in the kidneys in health and disease must be further explored.

Acknowledgements. M.Z. is funded by a NIH training grant to BIDMC (532T DK07760) and DK62364 (to Raghu Kalluri). I thank Dr R. Kalluri for his mentorship and the Kalluri laboratory for their help and support.

Conflict of interest statement. None declared.

References

Measurement of microalbuminuria – what the nephrologist should know

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**Keywords:** microalbuminuria; urinary albumin excretion; hypertension; diabetes; cardiovascular risk

**Introduction**

During the last few years, a subtle increase in urinary albumin excretion (UAE) not detectable by routine methods, so called microalbuminuria, has been identified as a prognostic marker for renal and/or cardiovascular risk in diabetic and non-diabetic subjects [1]. Consequently, assessment of microalbuminuria is now recommended as a risk stratification strategy not only in diabetic subjects, but also in the management of hypertensive patients [2–5]. In order to make the best clinical use of UAE, the physician who measures UAE should know several facts:

a. what kind of albumin molecules are present in the urine, and which methods are most suitable for assessing each of them;
b. what method of urine sampling is recommended and how should one interpret the UAE values;
c. how can one reduce the variability of the UAE estimate and
d. how should one evaluate the results and manage the patient based on the results of UAE determination.

Albumin is an electronegative serum protein with a molecular mass of 66 349 Da. After glomerular filtration, part of the albumin is reabsorbed by tubular epithelial cells. Proteases split the albumin molecule into fragments, some of which back-leak into the tubular fluid [6]. In addition, albumin can reach the urine from an inflammatory lesion at any site from the renal pelvis to the urethra. In the absence of inflammation in the urinary tract, intact albumin of glomerular origin is the major source of albumin in the urine and only a small amount of small albumin fragments are present.

**Methods to measure urinary albumin**

Albumin can be detected by several methods based on precipitation (boiling, sulphosalicylic acid), dye-binding (biuret, tetrabromphenol, albumin blue 580) or immunologic detection (radioimmunooassay, nephelometry, test-strip) (Table 1). While the immunoreactive methods estimate only complete albumin molecules recognized by antibodies, peptide fragments of albumin can be assessed by dye tests and specific spectrophotometry [7,8]. The immunologic methods are most frequently used for clinical purposes, not only because they are easy to use at relatively low cost, but also because they are able to detect small amounts of albumin in the range defined as microalbuminuria, i.e. <200 mg/l.