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

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Introduction

Several recent studies have demonstrated unequivocally that administration of bone morphogenic 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, hematopoietic 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
circuit 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-2). 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]. Consequentially, thinking evolved that
lack of endogenous BMP-7 expression could be compen-
sated 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]. Further-
more, in the setting of chronic renal disease, BMP-7
expression does not appear to correlate with progress-
ion 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 respon-
sible 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 pro-
peptide 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 associa-
tion 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
(uterine 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 I\(^{125}\)-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.

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References
Measurement of microalbuminuria – what the nephrologist should know

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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, sulphasalicylic acid), dye-binding (biuret, tetrabromphenol, albumin blue 580) or immunologic detection (radioimmunoassay, 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.