

Roles and regulation of bone morphogenetic protein-7 in kidney development and diseases

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Abstract

The gene encoding bone morphogenetic protein-7 (*BMP7*) is expressed in the developing kidney in embryos and also in the mature organ in adults. During kidney development, expression of *BMP7* is essential to determine the final number of nephrons in and proper size of the organ. The secreted BMP7 acts on the nephron progenitor cells to exert its dual functions: To maintain and expand the progenitor population and to provide them with competence to respond to differentiation cues, each relying on distinct signaling pathways. Intriguingly, in the adult organ, BMP7 has been implicated in protection against and regeneration from injury. Exogenous administration of recombinant BMP7 to animal models of kidney diseases has shown promising effects in counteracting inflammation, apoptosis and fibrosis evoked upon injury. Although the expression pattern of *BMP7* has been well described, the mechanisms by which it is regulated have remained elusive and the processes by which the secretion sites of BMP7 impinge upon its functions in kidney development and diseases have not yet been assessed. Understanding the regulatory mechanisms will pave the way towards gaining better insight into the roles of BMP7, and to achieving desired control of the gene expression as a therapeutic strategy for kidney diseases.

Key words: Bone morphogenetic protein-7; Therapeutics; Kidney; Development; Nephron progenitor cells; Disease; Regeneration; Chromatin conformation; Gene expression; Gene regulation

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Core tip: Bone morphogenetic protein-7 (BMP7) plays crucial roles in both the development and regeneration of the kidney. The functions and mechanisms of this protein have been clarified extensively for these processes in the fetus and adult kidney. However, the functional differences of BMP7 secreted from different sites in the kidney remain

undefined. We propose that uncovering the regulatory mechanism underlying *BMP7* expression will help to solve that issue. Moreover, those data should pave the way towards development of a novel therapeutic strategy for kidney diseases *via* hyperactivation of the endogenous action of *BMP7*.

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INTRODUCTION

Bone morphogenetic protein-7 (*BMP7*) belongs to the transforming growth factor- β (TGF β) superfamily. It was first identified and cloned as a human homolog of the bovine osteogenic proteins, and designated as the osteogenic protein-1 (OP-1)^[1,2]. Knockout mouse models of the *BMP7* gene were reported subsequently. Most strikingly, these models exhibited severe retardation of kidney development and died soon after birth due to renal dysplasia. Additionally, these mice exhibited anophthalmia and polydactyly in the hind limbs. Other phenotypic changes were also observed in ribs and craniofacial bones, but the effects were not fully penetrant^[3-5]. Since then, the roles of *BMP7* in the various stages of kidney development have been extensively studied. Interestingly, *BMP7* was found to be expressed not only during embryogenesis but also in the adult organ^[6-8]. Series of studies have now shed light on the protective and regenerative functions of its expression in the mature kidney^[9]. Furthermore, exogenous administration of *BMP7* and its mimetic has been considered as a promising therapeutic strategy for treatment of severe kidney diseases^[10].

Despite the original implication of an osteogenic property for *BMP7*, the *BMP7* knockout mice showed a severe phenotype in the kidney. This finding clearly illustrated that the function of the gene is critically determined by its expression pattern. In support of this, *Bmp4* under the control of the *BMP7* locus rescued the loss of the developmental function of *BMP7* in the kidney in mice^[11]. Thus, uncovering the regulatory mechanism for *BMP7* will be pivotal for gaining a better understanding of its functional roles and to developing therapeutic applications based upon it. In accordance with this perspective, in this review we first summarize the current knowledge regarding the function of *BMP7* in kidney development and diseases, after which we provide an overview of the recent findings in the regulation of its expression, finally discussing the future directions that will most likely advance the knowledge and clinical applications of this field.

BMP7 IN EMBRYONIC KIDNEY DEVELOPMENT

During embryonic development, *BMP7* is expressed in multiple tissues including the kidney, eyes, heart, limbs, forebrain, branchial arches, bones and cartilage^[6,12,13]. In the mouse, expression in the developing kidney appears first in the Wolffian duct, at embryonic day (E)9.5, and persists in the ureteric bud evaginated from the duct^[6]. At E11.5, *BMP7* expression appears in the condensing mesenchyme that is induced by the ureteric bud. Slight expression is found in the uninduced metanephric mesenchyme as well^[12]. At E13.5, the expression area extends to the pretubular aggregates and others derived from the condensed mesenchyme, including the comma-shaped and S-shaped bodies, the distal tubules and the podocytes of the developing glomeruli^[6,12,14], although expression in the comma-shaped and S-shaped bodies and the distal tubules was found by some of the studies to be very weak or absent^[4,14]. By E16.5, when the ureter has developed substantial branching, the expression in the ureteric epithelium becomes weaker in the medullar region, while its expression in the condensed mesenchyme in the nephrogenic zone of the developing kidney remains robust. Podocytes continue to strongly express *BMP7* after their folding in glomeruli^[3,4,12,13,15].

In mice, development of the kidney is severely retarded in the absence of *Bmp7*. In addition to the mutant kidney being smaller in size at birth, the number of nephrons is greatly reduced. These effects are accompanied by abnormal expansion of collecting ducts, which are interspersed by stromal cells and extracellular matrix. Mesenchymal stem cells and glomerulogenesis are also absent in the mutant kidney^[3,4].

These developmental defects appear as early as E12^[4]. As stated, the size of the mutant kidney is smaller than that of the control kidney, with the condensation of the mesenchyme appearing reduced at E12.5, although formation of pretubular aggregates was also observed^[4]. At E14.5, cessation of nephrogenesis becomes apparent with loss of mesenchymal populations in the cortical region^[3,4]. However, the comma-shaped and S-shaped bodies are present at this stage^[3,4] and the ureteric buds are branched^[3]. Moreover, the expression of marker genes, such as *Pax2*, *Pax8* and *Wnt4*, seems more or less normal in the two lineages, as long as the corresponding structures are present^[3,4].

These results suggested that the initial reciprocal inductive interaction between the ureteric epithelium and the metanephric mesenchymal cells takes place in the absence of *BMP7*^[3]. Studies using terminal deoxynucleotidyl transferase (TdT) dUTP nick-end labeling (commonly known as the TUNEL assay) showed massive apoptosis in the uninduced metanephric mesenchymal cells occurring from E13.5 to E14.5, explaining the

loss of the cell population that was observed in the mutant kidneys^[4,12]. Thus, BMP7 is a survival and/or proliferative factor that acts to maintain and expand nephron progenitor cells, the loss of which leads to severe retardation of kidney development^[3,4,12].

The molecular mechanisms that underlie the roles of BMP7 in kidney development have been uncovered by recent studies^[16]. The collective BMPs are known to activate SMAD1, SMAD5 and SMAD8 transcription factors, which are associated with receptors of BMPs and are phosphorylated upon their binding. Following phosphorylation, these SMADs form a complex with SMAD4, which then binds to *cis*-regulatory regions to activate target genes. On the other hand, BMPs can also activate the mitogen-activated protein kinase (MAPK) pathway, mediating their downstream effects^[17].

An *in vitro* study using a primary culture system of nephrogenic progenitor cells revealed that the proliferative role, but not the survival role, of BMP7 in this cell population is dependent on activation of the jun N-terminal kinase (JNK)-MAPK pathway^[18]. Mice with knockout of the *Trps1* gene, which encodes the trichorhino-phalangeal syndrome-1 zinc-finger transcription factor, show reduced epithelialization of mesenchymal progenitor cells^[19]. Interestingly, expression of *Trps1* in the kidney is dependent on BMP7, *via* p38 MAPK activation^[19]. Therefore, a part of the kidney-related phenotype of the *Bmp7*-null mice should be due to the deficiency in *Trps1* activation^[19].

An additional role of BMP7 involving the SMAD pathway was recently reported^[20]. In the nephrogenic zone, the progenitor populations are partitioned into two distinct compartments: One expressing detectable levels of both *CITED1* and *Six2* expression, and the other of *Six2* only. Importantly, the *Six2*-only compartment is responsive to the canonical Wnt9/ β -catenin signaling, showing appropriate differentiation and epithelialization; meanwhile, the *CITED1*⁺/*Six2*⁺ compartment is refractory to it^[20]. It has been shown that BMP7 promotes the transition of nephron progenitor cells from the *CITED1*⁺/*Six2*⁺ compartment to the *Six2*-only compartment *via* the activation of the SMAD pathway. Thus, BMP7 has multiple roles both in the proliferation and differentiation processes of the metanephric mesenchymal progenitor cells, most likely involving distinct signaling pathways.

Use of the two distinct pathways, MAPK and SMAD, in kidney development has also been suggested by the findings from *in vitro* studies. In an *ex vivo* experiment, BMP7 was shown to control branching morphogenesis of the ureteric buds in a dose-dependent manner; specifically, low dosage of BMP7 was shown to induce morphogenesis, while high dosage was shown to exert an inhibitory effect^[21]. A subsequent study showed that while the low-dose BMP7 induced p38 MAPK signaling, the high-dose triggered the SMAD pathway, which in turn caused negative regulation of the MAPK activity^[22]. Such bimodal regulation might also take place in the developmental context. In this sense, it would be intriguing to understand how secretion of BMP7 from

different sites, particularly the ureteric bud or the metanephric mesenchyme, impinges on the differential functional roles in the developmental process. To date, however, this process has remained largely unstudied.

Bmp7 expression continues in the developing kidney, even after the stage when the knockout mice present the severe abnormality. Several studies have aimed to uncover its roles in these later stages. A mouse strain that expresses the Cre recombinase under the promoter of *Nphs2* was used to create a conditional knockout mouse in which *BMP7* has near-complete specific deletion in the podocytes of mature glomeruli^[23]. These mice presented with hypoplastic kidneys, and proximal tubules of markedly reduced size. Concomitantly, phosphorylation of p38 MAPK was significantly reduced in the proximal tubules^[23].

Interesting phenotypes were observed upon deletion of the *BMP7* alleles at E12.5, which was accomplished using a mouse line expressing an inducible Cre^[24]. Deletion after the early stage of nephrogenesis resulted in precocious maturation of glomeruli, as well as increased apoptosis of the progenitor cells. *In vitro* assays further showed that BMP7 inhibits epithelialization of the mesenchymal progenitor cells, which might explain the precocious maturation that was observed^[24]. These findings might appear to be contradictory to the above-mentioned model in which BMP7 is required for shifting the competence of the mesenchymal progenitor cells for the differentiation cue^[20]; however, at an early stage, the metanephric mesenchymal cells do not need the BMP7/SMAD pathway for differentiation^[20]. In fact, the *BMP7* knockout mice can develop nephron structures adequately up to E13.5. Therefore, the reduction of BMP7 at E12.5 might guarantee or even accelerate the early phase of nephron formation. The late stage formation observed in the deletion mice might be attributed to the remaining expression of *BMP7* (around 10% as compared to the controls) after the induction by tamoxifen, particularly as BMP7 exhibits dose-dependency in induction of the downstream cascades^[22]. However, further studies are necessary to clarify this issue.

Overall, BMP7 mainly acts to determine and balance the fates of the mesenchymal progenitor cells, between proliferation and differentiation, to determine the final size of the mature kidney. Distinct pathways are utilized for these different roles. Although the mechanism to switch between the different pathways is largely unknown, dose-dependent regulation might contribute, at least partially. Therefore, regulation of the expression of *BMP7* is expected to play a critical role in the developmental process, and this topic will be discussed later in this review.

BMP7 IN THE ADULT KIDNEY

Kidney-specific expression of *BMP7* persists in the mature organ of the adult^[7]. Its functional significance has been suggested by a series of studies. All the more,

exogenous administration of BMP7 to injured kidneys was also shown to have therapeutic effects, including prevention of fibrosis, inflammation and apoptosis. We first summarize, here, the findings regarding the latter, and then we discuss the endogenous role of the protein at the end of this section.

Systemic administration of recombinant BMP7 to a rat model of ischemia/reperfusion injury was first shown to enhance recovery after acute injury by suppressing inflammatory responses, apoptosis and fibrosis^[25]. In subsequent studies, the administration of BMP7 to a rat model of unilateral ureteral obstruction (UUO) using prevention protocols resulted in blunting of the development of injury^[26,27].

Renal fibrosis is considered as a hallmark of chronic kidney diseases, although functional benefits of fibrosis have been recognized recently^[28]. TGF β 1 is a key mediator of fibrosis in many tissues, including in the kidney in response to injury (reviewed in^[29,30]). Binding of TGF β 1 to its type II receptor, TBR2, triggers the receptor to activate the TGF β receptor type I (TBR1)-kinase, which in turn induces downstream cascades *via* phosphorylation of SMAD2 and SMAD3^[31]. On the other hand, ligand binding to the type I activin-like kinase (Alk) receptors and type II serine/threonine kinase receptors (BMPRII) for BMP7 activates SMAD1/5/8 for SMAD signaling^[31].

Roles of these signaling pathways in renal fibrosis were investigated in an *in vitro* model^[32]. Incubation of mouse distal tubular epithelial cells (NP1) with TGF β 1 led to induction of epithelial-to-mesenchymal transition (EMT) that was associated with nuclear localization of phosphorylated SMAD2/3^[32]. However, addition of BMP7 to this culture system reversed the EMT through phosphorylation and activation of SMAD1, which in turn transcriptionally up-regulated the expression of E-cadherin, an important adhesion molecule in epithelial cells^[32].

Based on these findings, the counteraction of BMP7 against the TGF β 1-induced EMT was further tested *in vivo*. Intraperitoneal administration of BMP7 to a mouse model of progressive chronic kidney injury with nephrotoxic serum nephritis (NTN) led to reversal of the renal pathology and to a decline in the mortality rate^[32]. The same group also showed that the BMP7 treatment could attenuate progression of chronic kidney fibrosis in two genetic mouse models, namely those of Alport's syndrome and lupus nephritis^[33].

Recent studies have revealed involvement of epigenetic regulation in the renoprotective effect of BMP7. *Rasal1*, the gene encoding rasGAP-activating-like protein 1, was shown to be aberrantly hypermethylated in the fibrotic condition that is induced by TGF β 1^[34]. Reversal of fibrosis by the administered BMP7 was also found to be associated with active removal of methylation at *Rasal1* *via* the 10-11 translocation enzyme-3 (Tet3)^[35].

In contrast to the above findings showing the therapeutic effects of BMP7, the function of the endo-

genously expressed molecule in the adult kidney has not been thoroughly assessed to date. This might be partly due to the technical difficulty of eliminating BMP7 specifically in the adult kidney and not in the developing kidney, so as to avoid the developmental arrest that otherwise leads to death. However, several studies have demonstrated the pivotal role of endogenously expressed BMP7 in protection of the kidney from injuries.

Uterine sensitization-associated gene-1 (USAG1) is a BMP antagonist^[36-38], and is abundantly expressed in the adult kidney^[38]. The *Usag1* knockout mice show resistance to apoptosis and fibrosis, and a down-regulation in the expression of inflammatory genes, all of which were reinduced upon administration of neutralizing antibodies against BMP7^[39]. Thus, BMP7 appears to play a renoprotective role endogenously in the kidney, which is negatively regulated by USAG1. As mentioned above, fibrosis and inflammatory responses upon kidney injury seem to have beneficial effects as well for the renal function, serving to sustain the overall structure of the kidney^[28]. In this sense, USAG1 and BMP7 might cooperatively serve to balance the progression of fibrosis, and titration of these two proteins in the progression of kidney diseases might be an exciting approach for therapeutics.

Kielin/chordin-like protein (KCP) is, on the other hand, an enhancer of BMP signaling. Interestingly, the knockout mouse of the encoding gene develops susceptibility to kidney injury, further demonstrating the protective role of BMP7 in the adult kidney^[40].

Activin-like kinase 3 (Alk3) is one of the three type I receptors for BMP7^[31,41]. During the progression of kidney injury, *Alk3* is up-regulated, while the other receptor genes, *Alk2* and *Alk6*, are not. Loss of *Alk3* leads to more severe fibrosis and inflammatory response upon NTN-induced chronic kidney fibrosis, further supporting the theory that BMP7 exerts renoprotective functions through binding to *Alk3* endogenously^[41]. Furthermore, the small peptide agonist THR-123 exhibits therapeutic effects when applied to different models of kidney injuries, through its interaction with *Alk3*^[41].

REGULATION OF *BMP7* EXPRESSION IN THE EMBRYONIC AND ADULT KIDNEY

In the mouse embryo, *BMP7* is expressed not only in the kidney but also in various other tissues. A recent study showed that expression in extra-nephrotic domains is mostly regulated by long-range enhancers that activate gene expression in a tissue-specific manner around the locus^[42]. The previous *in vivo* studies had identified some of the enhancers capable of inducing reporter gene expression in the developing kidney. One such element is located in intron 1 of *Bmp7*, which is strongly conserved in tetrapods^[43]. When heterologously linked to the lacZ reporter under control of a minimal promoter sequence (Hsp68lacZ)^[44], the element induced lacZ expression in the Wolffian

duct, mesonephric tubules, ureteric bud and collecting duct, from E9.5 until E12.5, but not in the metanephric mesenchymal lineage. Of note, this expression could not be recapitulated by the orthologous sequence in *Xenopus tropicalis*. A lacZ reporter construct under control of the endogenous promoter of *BMP7* was also injected into mouse embryos together with the 4-kb upstream and the 3-kb downstream regions of the transcription start site (TSS) at each side, retaining the endogenous context^[43]. This entire construct was able to induce reporter expression in the nephrogenic mesenchymal regions. Interestingly, however, none of the separated individual elements covering the upstream and downstream regions was able to drive the expression when linked to Hsp68lacZ^[43].

Chromatin immunoprecipitation coupled with high-throughput sequencing (ChIP-seq) was performed in Six2⁺ nephron progenitor cells^[45]. A region located at 98-kb downstream of the TSS of *BMP7* was found to be co-bound by Six2 and β -catenin. When tested *in vivo*, this element induced gene expression in a compartment of Six2⁺ renal vesicles, a part of the *BMP7* expression domain^[45]. The ChIP-seq also identified a Six2 binding site in intron 1, an evolutionarily conserved region adjacent to the intron 1 enhancer, though a reporter construct including this region did not show enhancer activity in the developing kidney^[43]. These results suggest that *BMP7* expression in the different compartments of the developing kidney, notably the ureteric bud and the metanephric mesenchyme, might be regulated by a distinct set of enhancers that are active in the respective domains, possibly interacting in a cooperative manner with each other. However, to determine whether or not these elements actually contribute to the *BMP7* expression in the kidney, testing by deletion of the respective regions should be performed in future studies.

Bmp7 is highly and specifically expressed in the adult kidney under normal physiologic conditions^[7]. The main expression domains are the ureter, collecting duct, thick ascending limb, distal convoluted tubules and podocytes in the glomerulus^[46,47]. *BMP7* expression in these cell types might also be regulated by *cis*-regulatory enhancers that are embedded around the locus. However, to date, no such enhancer elements have been described for the expression in adult kidney. Acetylation of K27 of histone H3 (H3K27ac) is associated with enhancer activity of the marked regions. We compared released data from the ENCODE project of ChIP-seq for H3K27ac in kidney tissues at different time points, ranging from E14.5 to the adult stage^[48]. In Figure 1, the regions with peaks are more or less common between different stages, but there are some striking differences. Notably, the region of the intron 1 enhancer is highly acetylated at the embryonic stages, but the mark is almost diminished for the adult kidney. These data might suggest that different sets of *cis*-regulatory regions contribute to the expression at different stages.

Once the kidney is damaged, the levels and sites of *BMP7* expression are dynamically altered^[15,47]. The injuries cause dramatic response to the cellular states in the kidney *via* the inflammatory response and other signaling cascades, such as that involving TGF β 1. Such responses are expected to lead to alteration of epigenetic states around the *BMP7* locus; as a result, *BMP7* expression would be dynamically regulated. In the following passages of this review, we review the expression dynamics of *BMP7* in several kidney disease models, as reported in the literature to date, and discuss how they are regulated.

In the kidneys of the ischemia/reperfusion rat model, *BMP7* expression dramatically decreases soon after reperfusion, particularly in the outer medulla and glomeruli^[47,49]. This reduction might be related to the up-regulation of TGF β 1. The immunostain signal of BMP7 increases in proximal tubular epithelial cells, which are devoid of its expression in the normal situation^[50]. Similar up-regulation of *BMP7* in the proximal tubular epithelial cells following ischemia was confirmed in a mouse model^[51]. Proximal tubular epithelial cells from human patients with proteinuric nephropathies also showed up-regulation of *BMP7* as compared to that in healthy controls^[52]. In an experimental model of diabetic nephropathy, *BMP7* expression was decreased^[53], which might be due at least partly to the concomitant increase in *Tgfb1* expression. A tubular injury induced by folic acid resulted in reduced *BMP7* expression at first, but was followed by a gradual recovery of expression in the regenerative phase^[15]. In the cisplatin nephrotoxicity model, however, no or only a subtle increase in *BMP7* expression was scored^[15,54].

It has long been postulated that TGF β 1, which is an inducer of the fibrotic response upon injury, down-regulates *BMP7* expression (as discussed above). On the other hand, MyoR has been implicated in the activation of *Bmp7*^[54,55]. However it has not been assessed adequately to conclude whether these effects are direct or not.

Epigenetic regulation has been studied to understand the direct linkages between various cellular states and *BMP7* expression. In TGF β 1-induced EMT in human renal proximal tubular epithelial cells, *BMP7* is slightly down-regulated^[56]. Treatment with trichostatin A, a histone deacetylase (HDAC) inhibitor, however, led to deposition of acetylated histones around the promoter of *BMP7* and to induction of its expression, thereby counteracting the fibrosis^[56]. Consistently, in an ischemia/reperfusion mouse model, down-regulation of HDAC5 was found to be involved in the activation of *BMP7* during the regenerative phase following injury, probably *via* acetylation of histones^[51].

Another layer of epigenetic regulation might also impinge on the expression of *BMP7* in kidney. The topological conformation of chromatin has recently been recognized as an important determinant of transcriptional regulation of genes. Particularly, a topologically-associating domain (TAD; a compartmentalized block of the genome,

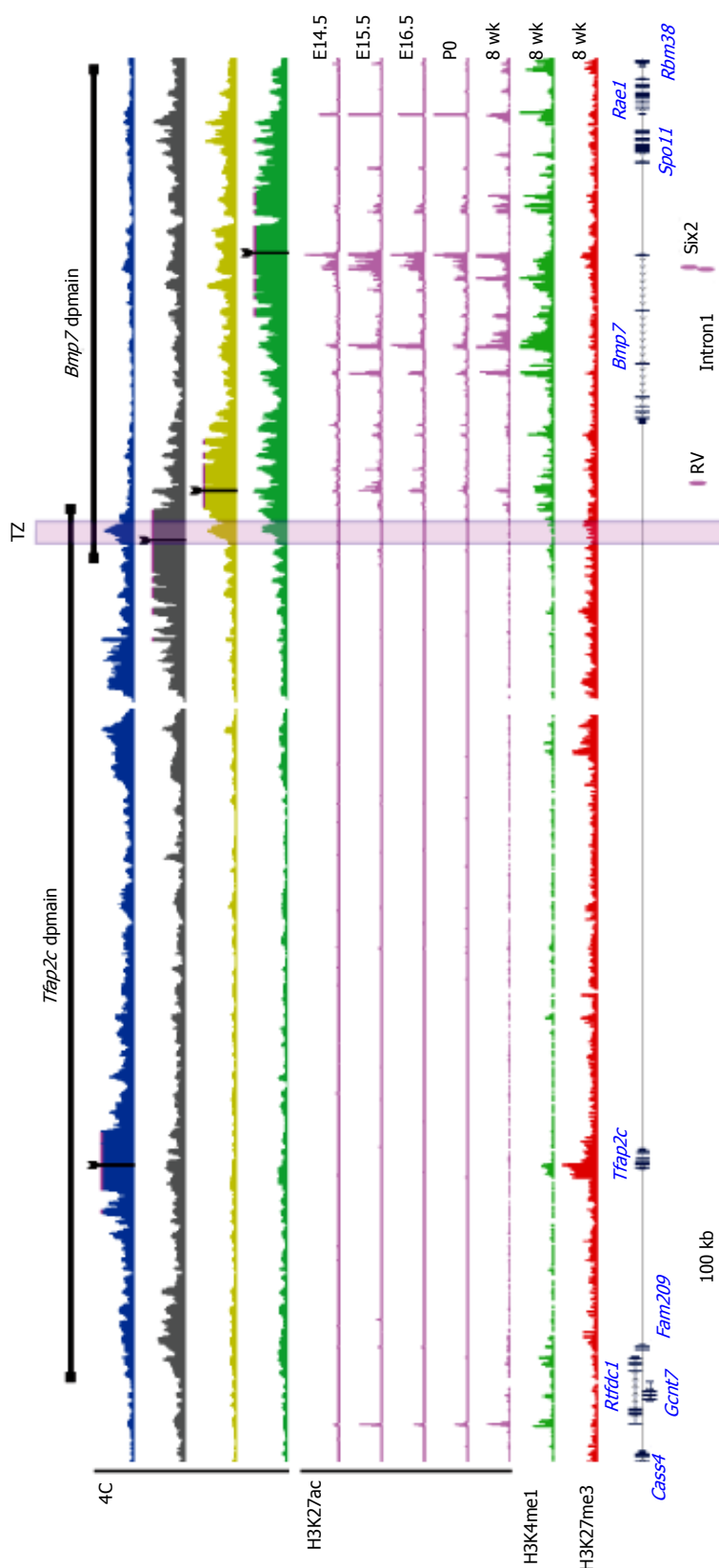


Figure 1 Landscape of the enhancers in the kidney and topological chromatin domains around the *BMP7* locus. Exons and introns of the genes within the locus (chr2: 172,250,000-172,850,000 mm9) are represented by blue boxes and arrowed dashes, respectively. Names of the genes are indicated above or below the respective boxes. The ChIP-seq signals of H3K27ac in the kidney tissue at different stages [E14.5, E15.5, E16.5, postnatal day (P)0 and 8-wk-old; indicated to the right] are shown with pink plots. H3K4me1 and H3K27me3 signals in the adult kidney are shown with green and red plots, respectively. The ChIP-seq data were obtained from the ENCODE project^[48]; the Data Coordination Consortium accession numbers are ENCSR703ZPF, ENCSR000CAF and ENCSR000CFP for H3K27ac, H3K4me1 and H3K27me3, respectively. Enhancer candidates are represented by pink ovals at the bottom: The RV enhancer is bound by Six2 and β -catenin, and induces reporter expression in the Six2-compartment of the renal vesicle^[45]; the intron 1 enhancer drives reporter expression in the developing ureteric buds^[43]; the Six2 binding site next to the intron 1 enhancer was identified by ChIP-seq^[45] but is not sufficient to induce gene expression in the developing kidney^[43]. Note that the H3K27ac mark around the intron 1 enhancer during embryogenesis diminishes in the adult stage. The chromatin domains identified by 4C-seq are shown at the top (indicated by whiskered lines), together with the actual results of the 4C-seq that are shown below^[42]. The viewpoints of the 4C-seq are indicated by arrows on the plots: Tfap2c promoter (blue), transition zone (TZ; gray), next to TZ in the BMP7 domain (yellow), and BMP7 promoter (green). The European Nucleotide Archive accession number of the 4C-seq data is ERP005557^[42]. The TZ between the two domains is indicated by the purple rectangle that spans the entire diagram.

in which the genomic regions preferentially associate with each other) was characterized as a ground-state structure that facilitates and constrains the interaction between enhancers and promoters of genes within it^[57-59].

Bmp7 is flanked by a large intergenic region, at

the other side of which a developmental gene *Tfap2c* is located (Figure 1). A recent study showed that the locus is conformationally partitioned into two adjacent domains, one for *BMP7* and the other for *Tfap2c*, by function of a region at the boundary, termed TZ^[42]. At

this locus, the action of enhancers is limited to genes located within the same domain that they belong to. In Figure 1, the TZ and 4C (circular chromatin conformation capture) plots that describe the physical domain structure were overlaid on the ChIP-seq map. It is apparent that the acetylation only extends within the *BMP7* domain and not to the neighboring one, underlining the importance of the topological structure for the regulation of *BMP7* in the kidney as well (Figure 1).

TADs seem to be more or less stable in different cell types. This might be due to the fact that CTCF, a ubiquitous DNA binding protein, greatly contributes to the formation of the domain structures^[57,60]. However, the topological structure is also a function of other epigenetic modifications, such as transcription of constitutive genes and polycomb group proteins^[57,61]. Indeed, the extent that enhancers can activate genes is sometimes different among different enhancers at the same locus^[42,59]. Therefore, it might be possible that the topological structure is subject to regulation for the dynamic expression of *BMP7* in response to kidney injuries.

CONCLUSION

BMP7 plays an important role in development and diseases of the kidney. In development, *BMP7* is critical both in proliferation and maintenance of the kidney's mesenchymal stem cells and in shifting their competence to respond to differentiation cues. Consequently, *BMP7* is a critical determinant of nephron numbers and the size of the organ. At the adult stage, *BMP7* is implicated in protection and regeneration of the kidney upon injury. Moreover, administration of *BMP7* and its mimetic exerts therapeutic effects in conditions of both acute kidney injuries and chronic kidney diseases.

Precise regulation of the *BMP7* gene is critical to its function in the kidney. At the embryonic stage, the major expression sites are metanephric mesenchyme and ureteric buds, two different lineages that interact with each other. Studies to date have indicated that the active locale of *BMP7* is the mesenchymal cells, rather than the ureteric epithelia, but the functional difference of the expression sites remains elusive. In this sense, it will be insightful to identify *cis*-regulatory elements that induce *BMP7* expression in the different cell populations and to test impacts of mutations in the elements on kidney development. The regulatory mechanisms of the adult kidney also remain elusive. Identification of the enhancers will provide insight into the still opaque role of *BMP7* in the adult kidney.

Understanding the epigenetic mechanism may not only clarify the dynamic regulation of the gene, but also open up a new avenue for therapeutics for kidney diseases through control of the expression of *Bmp7*. Different layers of epigenetic regulation, such as DNA methylation, histone modifications and higher-order chromatin conformation, almost certainly will bear a role in achieving delicate control of the gene's expression. Each of the layers represents a possible

target for therapeutics. Indeed, inhibition of HDAC has already shown a promising effect in augmenting *BMP7* expression in a TGFβ1-induced fibrosis model^[56]. Furthermore, recent advances in genome editing tools, such as the CRISPR/Cas9 system, might allow us to control epigenetic modifications, including higher-order chromatin conformation, in a locus-specific manner to optimize the gene expression^[62]. To this end, it will be beneficial to further deepen our understanding both of the role and regulation of *BMP7* in kidney development and diseases.

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