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BMP signaling in the nephron progenitor niche

Leif Oxburgh, Aaron C. Brown, Jennifer Fetting, and Beth Hill

Department of Molecular Medicine, Maine Medical Center Research Institute, 81 Research Drive, Scarborough, ME 04074, USA

Abstract

Bone morphogenic proteins (BMPs) play diverse roles in embryonic kidney development, regulating essential aspects of both ureteric bud and nephron development. In this review, we provide an overview of reported expression patterns and functions of BMP signaling components within the nephrogenic zone or nephron progenitor niche of the developing kidney. Reported in situ hybridization results are relatively challenging to interpret and sometimes conflicting. Comparing these with high-resolution microarray gene expression data available in Gudmap, we propose a consensus gene expression pattern indicating that essential components of both the Smad-mediated pathway and the Smad-independent mitogen activated protein kinase (MAPK) pathways are expressed in the nephron progenitor cell compartment and may be activated by BMPs, but that cortical interstitium may only be able to respond to BMPs through MAPK signaling. Localization of phosphorylated Smad transcription factors and studies of a BMP reporter mouse strain however indicate limited transcriptional responsiveness to Smad-mediated signaling in cap mesenchyme. An overview of genetic inactivation, organ culture, and primary cell studies indicates that BMP signaling may elicit two important biological outcomes in the nephrogenic zone: survival of the cap mesenchyme, and the physical segregation of interstitial and progenitor cell compartments. Ongoing studies using a novel primary cell system that establishes the nephrogenic zone ex vivo are pursuing the concept that the balance between Smad-mediated and Smad-independent responses to BMP ligand may underlie these distinct outcomes.

Keywords

Bone morphogenetic protein; BMP; Nephrogenesis; Nephron progenitor cell; Nephron differentiation; Nephrogenic zone

Introduction

The role of bone morphogenic protein (BMP) signaling in kidney development has been actively studied since the mid-1990s when it was found that genetic inactivation of *Bmp7* in the mouse causes severe renal malformations [1, 2]. Since these initial reports, studies have implicated BMP signaling in initiation of Wolffian duct formation [3], ureteric bud growth, morphogenesis and branching [4–9], nephron progenitor survival and proliferation [10–12], nephron-collecting duct fusion [13], nephron differentiation [14, 15], and glomerular capillary formation [16]. BMP functions in nephrogenesis have been thoroughly reviewed [17–19], and the purpose of this article is to focus on the role of BMP signaling in the life cycle of the nephron progenitor cell, highlighting recent work.

The metanephric kidney develops through a series of repetitive branching and inductive events between the collecting duct system, and the cap mesenchyme, or nephron progenitor cell population. This results in the centrifugal deposition of 10,000–12,000 nephrons per kidney between E11 and approximately P3 when nephrogenesis arrests in the mouse [20, 21]. The nephrogenic zone (Fig. 1a, b) located at the periphery of the kidney, supplies nephron progenitor cells to the radiating collecting duct tips that induce their epithelial differentiation. The self-renewal of nephron progenitors is regulated within this intriguing progenitor niche, as is the timing of their differentiation. Three major cellular compartments are represented within the nephrogenic zone: the collecting duct tip, the cap mesenchyme (nephron progenitor cells), and the cortical interstitium (Fig. 1b). Fate-mapping studies have determined that these three cell types belong to separate lineages, and that mixing of the three distinct compartments during nephrogenesis *in vivo* either does not occur, or is extremely uncommon [22–26]. Lineage marking of the odd skipped related 1 (*Osr1*) expressing field of cells within the intermediate mesoderm prior to appearance of the metanephric anlagen show that these cells give rise to both cortical interstitial cells and to cap mesenchyme cells prior to E11.5, approximately the time at which nephrogenesis commences. From this point onward, *Osr1* expressing cells give rise only to cap mesenchyme, demonstrating that segregation of nephron progenitor and interstitial lineages has occurred by the onset of nephrogenesis [27]. Extensive communication between the collecting duct, cap mesenchyme, and cortical interstitial compartments has been reported. For example, the glial cell line derived neurotrophic factor (GDNF) growth factor produced by nephron progenitors activates the c-Ret receptor on the collecting duct tip epithelium promoting growth and branching [28–31], while Wnt9b from the collecting duct promotes cap mesenchyme induction [32]. Furthermore, a retinoic acid signal from the cortical interstitium regulates expression of c-Ret in the collecting duct [33, 34], thus regulating collecting duct branching and growth and secondarily also nephron differentiation. In addition to receptor tyrosine kinase, Wnt and retinoic acid, BMP plays an important role in the control of progenitors within the nephrogenic zone. Genetic inactivation studies demonstrate that *Bmp7* is essential for the maintenance of nephron progenitor cells [1, 2], and in the absence of this growth factor kidney development is prematurely arrested.

BMP signaling is initiated upon ligand binding to a cell surface receptor complex consisting of type I activin receptor like kinase (ALK) receptors and type II receptors (Fig. 1c). Seventeen distinct proteins are classified as BMP ligands [35], and two distinct groups of ligands have served as prototypes for signaling studies: the Gbb subgroup consisting of BMPs 5, 6, and 7, and the Dpp subgroup consisting of BMPs 2 and 4. Physical interaction studies show that distinct ligand type I receptor pairings have different binding affinities. For example, BMP4 binds the ALK3 receptor but not the ALK2 receptor, whereas BMP7 binds ALK2 and ALK6 with high affinity and ALK3 with intermediate affinity [36, 37]. Thus, the level of responsiveness of a cell to BMP ligands may be controlled by expression of BMP type I receptors. Numerous different extracellular modulators of BMP ligand activity associate with BMPs to amplify or antagonize this process [38]. The process of extracellular modulation of signaling is complex, with proteins filling both antagonistic and amplifying functions. For example, chordin physically associates with and antagonizes signaling by BMP4 [39]. The protein twisted gastrulation associates with this complex, allowing diffusion within the extracellular space of the non-signaling BMP4:chordin:twisted gastrulation complex [40]. Upon metalloprotease cleavage of the complex, BMP4 ligand can bind receptor and initiate signaling. Extracellular complex formation can thus temporarily antagonize BMP signaling, preserving the ligand and allowing it to diffuse over significant distances. A further modification of this process involving the Crossveinless 2 (CV2) protein results in amplification of BMP signaling. CV2 associates through its vWf domain with heparan sulphate proteoglycans at the surfaces of cells in which it is produced [41]. CV2 has a high affinity for BMP4:chordin:twisted gastrulation complexes, trapping these and

concentrating ligand at the cell surface [42]. Upon metalloprotease cleavage, ligand is liberated, associating with cell surface receptors and eliciting signaling. In addition to its signal-amplifying activity, CV2 displays an antagonistic activity similar to that of chordin when complexed with BMP alone and it can therefore be considered a bimodal regulator of BMP signaling.

Two main intracellular signaling cascades are elicited by BMP receptor activation. Most thoroughly understood is the activation of the Smad transcription factors, which translocate to the nucleus and modulate gene transcription, often in conjunction with other transcription factors [43]. This pathway contains components that are specific for BMP signaling, and its activation can therefore be relatively easily monitored, for example through the detection of nuclear accumulation of BMP-specific Smads [44]. The second major signaling cascade to be activated is the MAPK pathway. Specifically, the TGF β -activated kinase TAK (MAP3K7) associates with BMP receptors in complex with its molecular partners XIAP and TAB [45–47]. Upon receptor binding by BMPs, TAK phosphorylates p38 and Jun N-terminal kinase (JNK), which in turn mediate activation of transcription factors such as C-JUN, ATF2, and E26 avian leukemia oncogene 1 (ETS1). Whether Smad and MAPK pathways are independently or coordinately activated by BMP signaling is unclear, but studies suggest redundancy and even codependence between the two, indicating that the latter does occur [48, 49].

BMP signaling within the nephrogenic zone

Ribonucleic acid (RNA) analyses of ligands [50, 51], receptors [9, 52], Smad transcription factors [53], and extracellular modifiers [11] have revealed complex expression patterns in the nephrogenic zone. Perhaps due to low levels of expression of many pathway components, results of published in situ hybridization analyses have often been weak, and sometimes conflicting. In Fig. 1d we propose a consensus expression pattern of pathway genes based on a comparison of published in situ hybridization studies and the high-resolution microarray data in Gudmap [54]. The expression pattern proposed for TGF-beta activated kinase (TAK) is based on a comparison of immunohistochemical detection [55] and Gudmap data. From these analyses, it appears likely that the collecting duct tip, cap mesenchyme, and nascent nephron are competent to respond to locally produced BMPs. To ascertain whether this in fact occurs, two independent lines of inquiry have been pursued. First, immunohistochemical localization of phosphorylated Smad1/5/8 reveals some nuclear accumulation primarily in cells of the nascent nephron and collecting duct trunk, but very little staining in cells of the cap mesenchyme or collecting duct tips [23, 56, 57]. An additional report contradicts these findings, showing nuclear accumulation of phosphorylated Smad in both cap mesenchyme and collecting duct tip [11]. These reports used the same antibody, and the cause of this discrepancy is unclear. One possible explanation may be differences in staining between batches of the polyclonal pSmad1/5/8 antiserum. To circumvent the problem of variability in immunodetection studies, a BMP reporter mouse was generated in which β -galactosidase expression is driven by a minimal promoter with associated Smad binding elements [57]. Studies of kidney development in this strain clearly shows a lack of BMP signaling in the cap mesenchyme and the collecting duct tip, but active signaling in collecting duct trunk and nascent nephron. Furthermore, scattered BMP-signaling cells were detected within the cortical interstitium. Results from transgenic reporter strains must of course be interpreted carefully due to the risk of uneven transgene expression because of genomic integration effects. Collectively, however, the BMP pathway expression analyses, phospho-Smad1/5/8 nuclear accumulation, and BMP reporter studies indicate very low levels of Smad-mediated BMP signaling throughout the nephrogenic zone of the developing kidney, although most of the cells within the zone appear competent to respond. One caveat to this interpretation is that the expression analysis

is based on RNA, and it is not certain that all pathway components are actually present at the protein level. For example, immunohistochemical studies of Smad1, 4, and 5 expression reveal very weak expression within the cap mesenchyme and collecting duct tip [58], contributing one possible explanation for the paucity of Smad-mediated signaling seen within these compartments.

The expression pattern of MAPK components within the nephrogenic zone has also been relatively difficult to define. However, immunohistochemical analysis [59] in conjunction with RNA expression data from Gudmap indicate that TAK is expressed throughout the nephrogenic zone. Immunoblot analysis of mixed populations of cap mesenchyme and cortical interstitial cells verifies this, and also shows that TGF-beta activated kinase binding protein (TAB) and X-linked inhibitor of apoptosis (XIAP), partners within the complex associating TAK with the BMP receptor, are expressed [12]. From this analysis, it is plausible that the entire nephrogenic zone is capable of responding in a Smad-independent manner to locally produced BMP ligands. The finding that the MAPK target transcription factors ATF2 and C-JUN are activated in the nephrogenic zone [12] support this interpretation.

Consequences of inactivation of BMP-signaling components in the nephrogenic zone

Ligands—Inactivation of *Bmp7* causes early depletion of cap mesenchyme cells within the nephrogenic zone and premature arrest of nephrogenesis at E15–E16 [1, 2]. Although few nephrons are formed in the *Bmp7* null kidney, the fact that they do differentiate strongly indicates that BMP7 function is limited to the progenitor cell compartment. Molecular marker analysis has not revealed any hallmark aberrations in the nephron progenitor cells, but increased apoptosis has been reported in presumptive nephron progenitor cells within the nephrogenic zone of the *Bmp7* null [2, 50]. Thus, BMP7 is proposed to function as a survival/maintenance factor for cap mesenchyme cells. This function does not appear to be specific to *Bmp7* as it can be replaced in vivo with *Bmp4* [60], raising the question of whether diffusion of BMP4 from the neighboring nascent nephron may promote the limited cap mesenchyme survival and proliferation that must occur to allow the nephrogenesis seen in the *Bmp7* null. Mutational analysis of *Bmp4* has not yielded any clues regarding its role in nephron progenitor maintenance due to the early and severe embryonic phenotype seen in the null, although roles in ureteric bud, collecting duct, and ureter development have been ascribed to this gene through analysis of the heterozygote and the conditional allele [9, 61].

BMP receptors—To date, only one experiment has been reported in which receptor gene expression has been inactivated in the nephrogenic zone. The *Hoxb7-cre;Alk3^{c/c}* inactivates *Alk3* throughout the ureteric bud and collecting duct, resulting in greatly reduced phosphorylation of Smad1/5/8 in this compartment and a pronounced defect in branching of the collecting duct [8]. Nephron development does not appear to be adversely affected by E18.5, although there is a deficiency in cap mesenchyme expression of *Pax2* in the *Hoxb7-cre;Alk3^{c/c}* kidney at E13.5.

Smads—Early and severe developmental phenotypes in *Smad4* and *Smad5* null animals have precluded analyses of nephrogenesis. To date, only conditional inactivation of *Smad4* has been reported [23]. Inactivation in the collecting duct using the *Hoxb7-cre* deleter does not result in any overt dysregulation of collecting duct branching and morphogenesis or nephron formation by E16.5. However, inactivation in the *Bmp7* domain of expression using *Bmp7-cre* results in a severe and complex phenotype in which cap mesenchyme cells mix inappropriately with cells of the cortical interstitium and nephrogenesis ceases by approximately E15. Superimposed on this, cell death can be seen within the nephrogenic zone, reminiscent of the *Bmp7* null phenotype.

Extracellular modifiers of BMP signaling—With the exception of the collecting duct tip, expression of Crossveinless (*Cv2*) overlaps with *Bmp7* in the nephrogenic zone (Fig. 1). Inactivation of *Cv2* results in an overall reduction in kidney size, with maintenance of a cap mesenchyme population and formation of nephrons similar to the wild-type [11, 62]. Analysis of kidney development in *Cv2;Bmp7* compound mutant animals reveals that the *Cv2* loss-of-function phenotype can be exacerbated by loss of a copy of *Bmp7*, indicating that CV2 may function as an amplifier of BMP7 signaling in cap mesenchyme. Indeed, immunostaining for nuclear accumulation of phosphorylated Smad1/5/8 reveals a decrease in cap mesenchyme signaling in the *Cv2* null that is exacerbated by loss of one allele of *Bmp7*. In this report, immunostaining is limited to the nephrogenic zone, and it is therefore not possible to relate the decrease in nuclear accumulation of phosphorylated Smad1/5/8 to levels seen in other analyses. Interestingly, *Cv2* mutant kidneys display mixing of cap mesenchyme and cortical interstitial cells similar to the *Bmp7-cre;Smad4^{c/c}* kidney, but no evidence of cell death in the nephrogenic zone is reported.

Integration of gene expression and inactivation data

Although both the expression analysis of BMP pathway components and the evaluation of their functions in the nephrogenic zone by gene inactivation studies remain very much works in progress, available data clearly shows that BMP7 produced by the cap mesenchyme signals in an autocrine or paracrine manner to promote the survival and maintenance of this population. Additionally, CV2 functions as an amplifier of BMP7 signaling in the cap mesenchyme. However, loss of *Cv2* does not result in any noticeable increase in cell death within the cap mesenchyme and instead causes inappropriate mixing of the cell populations found within the nephrogenic zone similar to that seen upon conditional inactivation of *Smad4* in the *Bmp7* domain of expression. This raises the possibility that there may be at least two distinct functions of BMP signaling within the nephrogenic zone: promoting survival and maintenance of nephron progenitor cells, and regulating segregation of cortical interstitium and cap mesenchyme within the nephrogenic zone.

Ex vivo studies of BMP function in the nephrogenic zone

Organ culture experiments have verified the role of the BMP7 ligand as a regulator of nephron progenitor survival [10, 51]. BMP7 reverses the cell death normally seen in isolated metanephric mesenchyme cultures from E11.5 kidneys, resulting in proliferation and growth of the mesenchyme culture [51]. Survival and proliferation is greatly enhanced by the addition of FGF2 [10]. Interestingly, the survival effects of these growth factors alone or in combination associate with a potent antagonistic effect on nephrogenesis in whole organ explants, with accumulation of undifferentiated cells in the nephrogenic zone. Although no definitive molecular marker analysis has been reported at the single-cell level, it appears from whole mount in situ analyses that the BMP7/FGF2 expanded population within the nephrogenic zone is comprised largely of cells expressing the cortical interstitium marker *Foxd1*. The interpretation of this finding remains challenging especially given the recent finding that *Foxd1* expressing cells are not part of the nephron lineage [25, 63].

To study cell-signaling events elicited by BMPs in the nephrogenic zone, we developed a culture method that establishes this zone ex vivo directly from the E17.5 kidney [12]. Using this system, we have established that BMP7 promotes proliferation of cap mesenchyme cells through the MAPK signaling pathway. Interestingly, phosphorylation of Smads1/5/8 can be detected in cap mesenchyme cells responding to BMP ligand stimulation, but activation of anticipated immediate early response genes such as the *Ids* is very weak suggesting an unorthodox transcriptional response [12, 57]. Both the TAK-JNK and Smad axes thus appear to be activated by BMP, but only inhibition of TAKJNK reduces the proliferative effect of BMP7. This implies that MAPK and Smad signaling have different biological

outcomes in this cell population. An interesting possibility is that proliferative/survival effects are mediated through MAPK signaling, whereas effects on segregation of cells within the nephrogenic zone are mediated through Smad.

Future perspectives

Although a great deal of attention has been devoted to understanding BMP signaling within the nephrogenic zone, both the mechanisms of signal transduction and its biological outcomes remain partially understood. The global gene expression analysis available in Gudmap has provided a rich source of candidate modifiers of signal transduction, and functional testing of these within the nephrogenic zone niche will allow us to build a more complete picture of pathway activation. The challenge of understanding biological effects regulated by BMP signaling in vivo is becoming more feasible with the development of new cap mesenchyme and cortical interstitium Cre deleter strains.

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Fig. 1.

Expression of BMP-signaling pathway components within the nephrogenic zone of the embryonic kidney. **a** The nephrogenic zone is located at the cortex of the E17.5 embryonic kidney, immediately under the capsule. **b** Further magnification of the boxed region in panel A shows the constituent cell compartments of the nephrogenic zone and an associated S-shaped body or nascent nephron. **c** Schematic representation of signaling pathways activated by BMP ligand binding to the type I/type II receptor complex at the cell surface. **d** Consensus gene expression pattern of BMP pathway signaling components based on comparison of reported in situ hybridization data and laser capture microdissection; microarray data from Gudmap. In addition to expression within the nephrogenic zone (CD, CI, CM), expression within the nascent nephron is shown. This structure develops from a renal vesicle through comma- and S-shaped bodies to form the tubular nephron. Abbreviations: *C* capsule; *CD* collecting duct; *CI* cortical interstitium; *CM* cap mesenchyme; *NZ* nephrogenic zone; *RV* renal vesicle; *SB* S-shaped body