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Bone morphogenetic protein signaling in nephron progenitor cells

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Abstract

Bone morphogenetic protein (BMP) signaling plays an essential role in many aspects of kidney development, and is a major determinant of outcome in kidney injury. BMP treatment is also an essential component of protocols for differentiation of nephron progenitors from pluripotent stem cells. This review discusses the role of BMP signaling to nephron progenitor cells in each of these contexts.

Keywords

Bone morphogenetic protein; BMP; Nephrogenesis; Nephron progenitor cell; Nephron differentiation; Renal repair; Embryonic stem cell differentiation

Embryonic nephron progenitor cells

Over the course of approximately 10 days in the mouse and 30 weeks in the human, the permanent metanephric kidneys are endowed with their full complement of nephrons. These are stereotypically arrayed, enabling establishment of the distinct regions within the mature organ essential for physiological function. In the rodent and human, iterative radial nephron formation ensures that this organ structure is attained, with mouse kidneys containing 10,000–15,000 nephrons and human kidneys 200,000–2,500,000 nephrons [1, 2]. Nephrons are formed by reciprocal induction between the epithelial ureteric bud (collecting duct) and metanephric mesenchyme. The ureteric bud grows into the metanephric mesenchyme, continually branching and inducing epithelialization of mesenchymal cells that become nephrons. Within the metanephric mesenchyme, cap mesenchyme located at the tips of the branching collecting duct has been accepted as the nephron progenitor cell population on the basis of morphological criteria, its capacity to form nephrons in isolation from the collecting duct, and its distinct molecular marker profile [3–6]. Lineage analyses using mouse strains expressing Cre recombinase under the control of genes expressed within the cap mesenchyme subsequently verified that this cell population indeed contains progenitors for all nephron segments [7–9].

Bone morphogenetic protein (BMP) signaling in embryonic nephron progenitor cells

Bone morphogenic protein signaling is initiated by ligand binding to cell surface serinethreonine kinase receptors, which can activate both SMAD and MAPK signaling cascades. We have previously summarized the expression of pathway components in

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nephron progenitor cells, which is schematized in Figure 1 [10]. BMP7 is uniquely expressed in cap mesenchyme and collecting duct tips [10 and references therein]. Genetic inactivation of *Bmp7* in the mouse causes premature arrest of nephrogenesis with depletion and cell death in the cap mesenchyme, suggesting that nephron progenitor cells require BMP7 for their proliferation and survival [11–13]. BMP7-initiated SMAD (BMP-SMAD) and MAPK (BMP-MAPK) signaling both occur in nephron progenitor cells [7, 14–17]. Type I BMP receptors are essential components of the membrane-associated complex through which BMP signaling is transduced, and expression data indicates that *Alk3* and *Alk6* are redundantly expressed in cap mesenchyme [18, 19]. In a recent report the type I BMP receptor *Alk3* was inactivated using *Rarb2-Cre*, which recombines in intermediate mesoderm and its cap mesenchyme and stromal cell derivatives, but not in the collecting duct [20]. *Rarb2-Cre;Alk3^{-flox}* embryos have hypomorphic kidneys, but interestingly this phenotype is distinct from the *Bmp7* null, lacking the hallmark cell death and premature depletion of the cap mesenchyme during metanephric development. Comparison of kidney size reveals significant differences at all stages, and analysis of intermediate mesoderm reveals a substantial reduction in the size of the nephrogenic field in the mutant. ALK3 is a high-affinity receptor for BMPs 2 and 4, and binds BMP7 with low affinity suggesting that BMP2/4 signaling through ALK3 determines the size of the nephrogenic progenitor field within the intermediate mesoderm [21, 22]. This is consistent with the essential role for ALK3 and BMP4 in mesoderm formation and patterning [23, 24]. Molecular marker expression within metanephric kidneys of *Rarb2-Cre;Alk3^{-flox}* mice reveals few qualitative differences from wild type controls indicating little or no requirement for *Alk3* in the cap mesenchyme. However, analysis of signaling in mutant kidneys at E13.5 shows reduced p38 MAPK but unchanged BMP-SMAD, suggesting some role for ALK3 in transducing MAPK signal downstream of BMP7, possibly redundantly with ALK6 and ALK2, although expression of the latter may be very modest. Further conditional compound inactivation of BMP receptors in cap mesenchyme will be required to define molecular pathways through which cap mesenchyme cells transduce the BMP7 signal.

BMP7 activation of a MAP3K7 (TAK1) – JNK pathway promotes nephron progenitor cell proliferation, providing an explanation for the premature exhaustion of nephron progenitor cells seen in the *Bmp7* null kidney [17]. However, recent studies indicate additional functions for BMP7 signaling in the cap mesenchyme. Detailed micro-anatomical studies have revealed that the cap mesenchyme is in fact composed of a series of sub-compartments with distinct molecular marker profiles [25]. A sub-compartment of the cap mesenchyme expresses CITED1 and lineage tracing using the *Cited1-creERT2;Rosa26R* strain has shown that these cells give rise to the entire cap and all cell types of the mature nephron, demonstrating that they are the highest-order progenitors so far identified within the cap [9]. Cap mesenchyme is induced by WNT9b secretion from the adjacent collecting duct [26]. A subset of the cap mesenchyme remains refractory to WNT9b induction, thus providing progenitors for subsequent rounds of nephrogenesis. The mechanism is incompletely understood, although requirements for expression of the SIX2 transcription factor and low-level WNT9b signaling have been reported [27, 28]. In primary culture studies we have found that CITED1-expressing progenitors are refractory to the inductive effects of β -catenin mediated signaling, indicating that this sub-compartment is in fact reserved from induction. Cells require BMP-SMAD signaling to exit the CITED1-expressing state to the next cap sub-compartment in which they are sensitized to the inductive effect of β -catenin [29]. Interestingly, a study of the physical association between SIX2 and β -catenin showed that while these proteins do associate in cap mesenchyme cells, they do not associate within the CITED1-expressing sub-compartment, indicating a significant difference between these cells in their transcriptional responses to WNT [30]. We propose that BMP7 not only acts as a maintenance and proliferation signal to the cap mesenchyme by initiating MAPK signaling, but also controls the sensitivity of nephron progenitor cells to WNT9b [17, 29].

The *Bmp7* null phenotype is consistent with this model: while cap mesenchyme cells display reduced survival, there is also a striking retention of CITED1-expressing cells within the cap mesenchyme and a lack of epithelializing nephron progenitor cells [29]. This bimodal function of BMP signaling is not well understood at the molecular level, and it remains to be determined if BMP-MAPK and BMP-SMAD are alternate outcomes downstream of BMP receptor activation, or if they might be complementary and occurring at different relative levels at different stages of progenitor cell differentiation. CITED1-expressing cells of the cap mesenchyme display low levels of phospho-Smad1/5, indicating weak or absent BMP-SMAD transduction [15]. However, the level of phospho-Smad1/5 rises once cells transition out of the CITED1-expressing compartment. Whether the strength of BMP-MAPK signaling displays an inverse relationship remains unknown. Molecular mechanisms for restriction of SMAD1/5 activation within the cap have not yet been defined. One possibility is that receptor phosphorylation of SMAD1/5 is antagonized, and thus that SMAD1/5 is transcriptionally inactive within the CITED1-expressing population. The gene encoding the phosphatase Dullard, which dephosphorylates BMP type I receptors and promotes proteasomal degradation of BMP type II receptors, is expressed in cap mesenchyme [31]. Mice in which *Ctdnep1* (dullard) has been inactivated specifically in this population display severe malformations of the juvenile kidney, but unperturbed embryonic kidney development [32]. This suggests either that restriction of receptor-mediated phosphorylation is not the mechanism that limits SMAD1/5 signaling in the cap mesenchyme, or that there is compensation by a redundant phosphatase. Further inquiry into the mechanisms by which BMP-MAPK and BMP-SMAD signaling cascades are initiated upon ligand binding, and studies of the interactions between BMP pathway components and other important regulators such as β -catenin and SIX2 are required to understand the specific molecular functions of BMP7 in the self-renewal versus differentiation decisions of nephron progenitor cells.

BMP in nephrogenic stem cell differentiation protocols

In the vertebrate embryo, intermediate mesoderm is the source of all kidney tissue and successful differentiation of stem cells to intermediate mesoderm *in vitro* is therefore a crucial step toward derivation of nephron progenitor cells. *Bmp4* is essential for mesoderm formation and patterning [23, 33]. The intermediate mesoderm is marked by genes including *Osr1*, *Pax2* and *Lim1* and studies in *Xenopus* and zebrafish show a requirement for BMP in expression of *Pax2* and *Lim1* [34–38]. Early studies using *Xenopus* met with partial success in the induction of intermediate mesoderm from pluripotent stem cells using defined media containing Activin A and retinoic acid (RA) and these factors also promote differentiation of Brachyury positive mesoderm from mouse ES cells [39–41]. Addition of BMP to Activin A and RA treated mouse ES cells enhances induction of mesoderm specific markers, potentially by blocking neuroectoderm differentiation [42, 43]. In addition, BMP7 enhances the capacity of ES cell derived embryoid bodies to integrate into polarized tubules when recombined with developing kidney rudiments [41]. In contrast to BMP7, BMP4 completely inhibited the ability of mouse embryoid bodies to contribute to tubules suggesting a significant difference in biological function between these two ligands. BMP4 promotes expression of both hematopoietic and intermediate mesoderm markers, whereas BMP2 and BMP7 treatment promote intermediate mesoderm marker expression only, providing further support for a difference between BMP ligands *in vitro* [44]. This was not anticipated based on functional studies conducted *in vivo*, and there may be important qualitative differences between these recombinant proteins that affect signaling outcomes, such as differences in half-life [45]. BMP7 has also been shown to specifically enhance expression of metanephric mesenchyme markers such as *Pax2* and *Wt1* in differentiated mouse ES and iPS cells, whereas the BMP antagonist Gremlin instead promotes neuronal differentiation [40]. A comparison of directed differentiation in human ES cells found that BMP4 and 7 promoted

intermediate mesoderm differentiation similarly. However, later during the culture period significant differences in the expression of *Osr1*, *Wt1* and *Lim1* were seen, suggesting important differences in the capacities of these ligands to induce nephron progenitor cells [46]. Signaling elicited by BMP in stem cell differentiation protocols has yet to be characterized, but extrapolating from *in vivo* studies, signaling amplitude is predicted to be an important factor. In the chick, both low and high intensity BMP signaling promote expression of the intermediate mesoderm genes *Osr1*, *Pax2* and *Lim1* through activation and inhibition of repressive activity in somatic mesoderm and lateral plate, respectively [47]. In the chick, nuclear localized phospho-SMAD1 is present in the intermediate mesoderm demonstrating that BMP is acting through the SMAD pathway [48]. Supporting a role for BMP-SMAD in intermediate mesoderm specification, it was recently found that nodal-like signaling promotes intermediate mesoderm formation by modulating BMP-SMAD activity [49]. Attempts to generate nephron progenitor cells from human embryonic stem cells or induced pluripotent cells similarly depend on BMP. In a two-stage differentiation protocol employing Brachyury-positive mesoendoderm, strong suppression of ectodermal and endodermal lineages was achieved during the first 2 days of culture with Activin A and the WNT agonist CHIR99021. In the second stage, replacement of Activin A with BMP7 for an additional 8 days produced greater than 90% OSR1+ cells, with many expressing the cap mesenchyme markers SALL1, PAX2 and WT1 [50]. Further culture of these OSR1+ cells for 7 days led to the expression of markers for nearly all cell types derived from the intermediate mesoderm including the nephric duct, ureteric bud, metanephric mesenchyme and interstitial and gonadal or adrenocortical cells. Given the diversity of cell types generated in this culture system, it is perhaps not surprising that they have limited capacity to generate 3-dimensional renal structures *in vitro* or *in vivo*. Modification of culture conditions to promote differentiation of a single renal progenitor subtype is a focus of intense activity. Interestingly, a recent study found that markers characteristic of nephron progenitor cells and renal epithelium could be promoted by culturing human ES cells on matrigel in renal epithelial cell medium with low concentrations of BMP2 and BMP7 together with Activin A [51]. Although BMP signaling is clearly necessary for the generation of renal subtypes from pluripotent stem cells, it appears that differentiation to specific intermediate mesoderm-derived progenitor subtypes may be strongly influenced by the dosage, oscillation of the treatment and manipulation of specific BMP signaling branches (SMAD versus MAPK).

Does BMP therapy in kidney injury target nephron progenitor cells?

Following unilateral nephrectomy and/or partial renal resection, remaining kidney tissue in adult humans, mice, and rats undergoes hypertrophic compensatory growth rather than *de novo* organogenesis [52]. However, despite this inability to form new nephrons, studies of the response to acute ischemia have demonstrated that the adult kidney has a strong capacity for *in situ* repair of damaged tubule epithelia [53]. More recent investigations of the ontogeny of the cells that reline the denuded epithelium following acute injury indicate that new cells arise from nephron epithelial cells that survived the initial insult rather than a dedicated adult nephron progenitor cell population [54]. BMP7 treatment of animals following ischemic injury reduces epithelial damage and accelerates healing, suggesting that this growth factor may act directly on surviving nephron epithelial cells to promote their regeneration [55]. Indeed, vigorous BMP-SMAD pathway activation can be seen in epithelial cells that are re-lining the nephron following acute injury [56]. Studies of BMP pathway components in the adult mouse kidney have revealed a complex regulatory system controlling endogenous BMP signaling in the kidney. Although the proximal and distal tubules express BMP2 and BMP7 respectively, signaling elicited by these ligands is limited by the expression of the extracellular antagonists CHRDL1 and USAG1 in the healthy kidney [56–59]. In addition to negative regulation in the healthy kidney, BMP signaling is

positively regulated in the injured kidney by the extracellular modulator KCP, which is upregulated in response to injury [60]. Thus, nascent nephron epithelial cells re-line nephron basement membranes in an environment highly conducive to BMP signaling. Inactivation of the ALK3 receptor in proximal epithelial cells sensitizes cells to injury and promotes fibrosis in an interstitial nephritis model, suggesting that the regenerative response may be dependent on epithelial expression of this BMP receptor [61]. In all, there is a compelling case for a pro-regenerative function of BMP signaling directly in epithelial cells that repair the nephron following injury. One mechanism by which BMP may accomplish this is by suppressing inflammatory signaling by epithelial cells. Gene expression studies show that BMP7 treatment suppresses IL6 and MCP expression, and injured kidneys from BMP7 treated mice display reduced inflammatory cell infiltration whereas mice in which *Alk3* has been inactivated in the proximal tubule show enhanced inflammatory infiltration [55, 57, 59, 61]. However, the effects of BMP are highly pleiotropic, and many other potential mechanisms remain to be explored using mice with conditional inactivation of BMP signaling components in nephron epithelial cells.

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Figure 1. Schematic of bone morphogenic protein (BMP) pathway components expressed in cap mesenchyme

BMP signaling is initiated by ligand binding to membrane associated type I (ALK3/6) and type II (BMPRII) receptors. Signaling is transduced through SMAD and MAPK pathways, which provoke distinct transcriptional responses.