ELSEVIER

Mechanisms of Development 122 (2005) 928–938

www.elsevier.com/locate/modo

Glypican-3 modulates inhibitory *Bmp2-Smad* signaling to control renal development in vivo

Sunny Hartwig[a,b], Ming-Chang Hu[a], Christina Cella[a], Tino Piscione[a,c], Jorge Filmus[d], Norman D. Rosenblum[a,b,c,e,*]

[a]*Program in Developmental Biology, Research Institute, The Hospital for Sick Children, Toronto, Canada*
[b]*Department of Physiology, University of Toronto, Toronto, Canada*
[c]*Division of Nephrology and Department of Paediatrics, The Hospital for Sick Children, 555 University Avenue, Toronto, Ont., Canada M5G 1X8*
[d]*Sunnybrook and Women's College Health Sciences Centre, University of Toronto, Toronto, Canada*
[e]*Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, Canada*

Received 23 November 2004; received in revised form 9 March 2005; accepted 27 March 2005
Available online 4 May 2005

Abstract

Renal branching morphogenesis, defined as growth and branching of the ureteric bud (UB), is a tightly regulated process controlled by growth factor-dependent tissue interactions. Previously, using in vitro models of branching morphogenesis, we demonstrated that BMP2 signals via its intracellular effectors, SMAD1 and SMAD4, to control UB cell proliferation and branching in a manner modulated by Glypican-3 (GPC3), a cell surface heparan sulfate proteoglycan. Here, we used loss-of-function genetic mouse models to investigate the functions of *Bmp2* and *Gpc3-Bmp2* interactions in vivo. Progressively greater increases in UB cell proliferation were observed in *Bmp2+/−*, *Smad4+/−*, and *Bmp2+/−*; *Smad4+/−* mice compared to *Wt*. This increased cell proliferation was accompanied by a significant increase in UB branching in *Smad4+/−* and *Bmp2+/−;Smad4+/−* mice compared to *Wt*. Reduction of *Gpc3* gene dosage also increased UB cell proliferation, an effect that was enhanced in *Gpc3+/−;Bmp2+/−* mice to an extent greater than the sum of that observed in *Gpc3+/−* and *Bmp2+/−* mice. Reduction of both *Gpc3* and *Bmp2* gene dosage enhanced cell proliferation in the metanephric mesenchyme compared to *Wt*, an effect not observed in either *Bmp2+/−* or *Gpc3+/−* mice. Phosphorylation of SMAD1, a measure of SMAD1 activation, was progressively decreased in *Gpc3+/−* and *Gpc3+/−;Bmp2+/−* mice compared to *Wt*, suggesting that *Gpc3* stimulates *Bmp2*-dependent SMAD signaling in vivo. These results demonstrate that BMP2-SMAD signaling, modulated by GPC3, inhibits renal branching morphogenesis in vivo.

Keywords: Glypican-3; BMP; SMAD; Cell proliferation; Branching morphogenesis

1. Introduction

Branching morphogenesis, defined as growth and branching of epithelial tubules during embryonic development, is fundamental to the development of many essential organs, including the mammalian kidney, lung, mammary gland, salivary glands, pancreas and seminiferous tubules, as well as the larval tracheal system in *Drosophila* [reviewed in (Davies and Fisher, 2002)]. In embryonic tissues, growth and branching of epithelial tubules is a highly coordinated process, regulated by both stimulatory and inhibitory pathways which signal between the branching tubules and surrounding mesenchyme [reviewed in (Shah et al., 2004)]. Renal branching morphogenesis is dependent upon reciprocal inductive interactions between the metanephric blastema, a mesenchymal tissue, and the epithelium-derived ureteric bud (UB). The collecting duct system of the kidney arises through the outgrowth and repeated branching of the UB and its daughter collecting duct cells (Potter, 1972; Saxen, 1987). UB growth and branching is preceded by highly localized increases in cell

* Corresponding author. Address: Division of Nephrology, The Hospital for Sick Children, 555 University Avenue, Toronto, Ont., Canada M5G 1X8. Tel.: +1 416 813 5667; fax +1 416 813 6271.
E-mail address: norman.rosenblum@sickkids.ca (N.D. Rosenblum).

0925-4773/$ - see front matter © 2005 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.mod.2005.03.007

proliferation at the UB tips suggesting that cell proliferation is an important morphogenetic mechanism controlling branching morphogenesis in vivo (Michael and Davies, 2004).

Glypican 3 (GPC3), a glycosyl phosphatidyl inositol (GPI)-linked heparan sulfate proteoglycan (HSPG), is essential for normal renal branching morphogenesis in vivo. *Gpc3* transcripts are expressed in both the mesenchymal and UB-derived tissue elements during embryonic renal development (Grisaru et al., 2001). In humans, mutations in *Gpc3* cause the X-linked overgrowth disease Simpson–Golabi–Behmel Syndrome, characterized in part by pre- and post-natal somatic overgrowth, and renal medullary cystic dysplasia (Pilia et al., 1996; Neri et al., 1998; Cano-Gauci et al., 1999). In mice, mutational inactivation of *Gpc3* causes renal medullary cystic dysplasia characterized by a reduction in the number of medullary collecting ducts, cystic malformation of medullary collecting ducts, and an increase in medullary interstitial extracellular matrix (Cano-Gauci et al., 1999). The appearance of these histopathologic abnormalities is preceded by selective overgrowth of the UB that is associated with a marked increase in UB cell proliferation (Grisaru et al., 2001), suggesting that abnormal regulation of UB cell proliferation contributes to the medullary dysplasia in *Gpc3* null mice.

Bone morphogenetic proteins (BMPs) belong to the TGF-β superfamily, a group of heparin-binding secreted growth factors that regulate cell proliferation, differentiation and organ development in many tissues including the kidney (Piscione et al., 1997; Miyazaki et al., 2000; Cain et al., 2005). Genetic *glypican-Bmp* interactions control development of non-renal tissues in flies (*dally-decapentaplegic*) and in mice (Jackson et al., 1997; Paine-Saunders et al., 2000; Fujise et al., 2003). In mice, *Bmp2* transcripts are expressed in condensed mesenchyme adjacent to the tips of the branching UB, consistent with a role for *Bmp2* in controlling cell proliferation and branching of the growing UB (Dudley and Robertson, 1997). However, homozygous deletion of *Bmp2* is early embryonic lethal, precluding analysis of the renal phenotype in these mice, while histologic analysis of *Bmp2*+/− heterozygote mice has been reported as normal (Zhang and Bradley, 1996). Thus, analysis of *Bmp2* mutant mice has not facilitated attempts to define the role of the *Bmp2* signaling axis in mouse kidney development. Recently, we demonstrated that a *Bmp2-Smad1* dependent signaling pathway inhibits UB branch formation and cell proliferation in isolated embryonic kidney explants and in the mIMCD3 culture model of collecting duct cell morphogenesis (Piscione et al., 1997, 2001; Gupta et al., 1999). While we have demonstrated that this inhibitory *Bmp2* pathway is regulated by *Gpc3* in these in vitro models, genetic *Bmp2-Gpc3* interactions have not been identified during kidney development in vivo.

In this work, we investigated the function of the inhibitory *Bmp2* signaling axis and genetic *Bmp2-Gpc3* interactions during renal development in vivo. We performed a high-resolution analysis of branch formation and cell proliferation during branching morphogenesis in vivo. *Bmp2* haploinsufficiency exerted no detectable effect on branch point formation. However, UB cell proliferation was increased in both *Bmp2*+/− and *Gpc3*+/− mice. A marked increase in UB cell proliferation greater than the sum of that detected in either *Gpc3*+/− or *Bmp2*+/− mice was observed in *Gpc3*+/−;*Bmp2*+/− mutants. *Gpc3*+/−;*Bmp2*+/− mice also exhibited increased mesenchymal cell proliferation, a defect not observed in either *Gpc3*+/− or *Bmp2*+/− mice. Our finding of decreased expression of phospho-SMAD1, an intracellular effector of BMP2, in *Gpc3*+/−;*Bmp2*+/− mice demonstrates that GPC3 modulates the activity of the BMP2 signaling pathway. Taken together, our results demonstrate that *Bmp2* and *Gpc3* interact to control renal development in vivo.

2. Results and discussion

2.1. The inhibitory Bmp2 signaling axis controls renal branching morphogenesis in vivo

We previously showed that exogenous BMP2 inhibits UB cell proliferation and branching in cultured kidney explants, and in the mIMCD-3 culture model of collecting duct cell morphogenesis (Piscione et al., 1997). Consistent with these observations, BMP2-mediated inhibition is decreased in collecting duct cells expressing mutant loss-of-function forms of the type I BMP2 receptor, ALK3 (Gupta et al., 2000; Piscione et al., 2001), or the intracellular BMP2 effector, SMAD1 (Gupta et al., 1999; Piscione et al., 2001). Taken together, these results implicate the inhibitory *Bmp2* signaling pathway in the control of embryonic renal development in vivo.

We examined *Bmp2* function in vivo by performing a high resolution analysis of UB branch point formation in mice heterozygous null for *Bmp2* or for its downstream effector *Smad4*, and in *Bmp2*+/−;*Smad4*+/− mice (Fig. 1). At E13.5, renal branching morphogenesis is well established and discrete branch points can be identified using the UB-specific marker DBA (Piscione et al., 1997). Despite a histologic normal phenotype (data not shown), quantitation of UB branch points demonstrated a 12% increase in UB branches in *Smad4*+/− mutants compared to *Wt* ($P=0.01$) (Fig. 1, Table 1). The increase in branch point formation in *Smad4*+/− mutants is consistent with the role of *Smad4* as the common mediator of both inhibitory TGF-β and BMP signaling during renal development (Shi and Massague, 2003). To determine a specific role of *Bmp2* in renal branching morphogenesis, we examined branch point formation in *Bmp2*+/− mice. *Bmp2* haploinsufficiency alone had no significant effect on UB branching ($P=0.59$). However, branch point number