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Bmpr1a is required for proper migration of the AVE through regulation of *Dkk1* expression in the pre-streak mouse embryo

Shigeto Miura [a], Ajeet Pratap Singh [a], Yuji Mishina [a,b,*]

[a] Laboratory of Reproductive and Developmental Toxicology, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709, USA
[b] Department of Biologic and Materials Sciences, School of Dentistry, University of Michigan, Ann Arbor, MI 48109, USA

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ABSTRACT

Here, we report a novel mechanism regulating migration of the anterior visceral endoderm (AVE) by BMP signaling through BMPRIA. In *Bmpr1a*-deficient (*Bmpr-null*) embryos, the AVE does not migrate at all. In embryos with an epiblast-specific deletion of *Bmpr1a* (*Bmpr1a*$^{null/flox}$; *Sox2Cre* embryos), the AVE cells migrate randomly from the distal end of embryos, resulting in an expansion of the AVE. *Dkk1*, which is normally expressed in the anterior proximal visceral endoderm (PxVE), is downregulated in *Bmpr-null* embryos, whereas it is circumferentially expressed in *Bmpr1a*$^{null/flox}$; *Sox2Cre* embryos at E5.75–6.5. These results demonstrate an association of the position of *Dkk1* expressing cells with direction of the migration of AVE. In *Bmpr1a*$^{null/flox}$; *Sox2Cre* embryos, a drastic decrease of WNT signaling is observed at E6.0. Addition of WNT3A to the culture of *Bmpr1a*$^{null/flox}$; *Sox2Cre* embryos at E5.5 restores expression patterns of *Dkk1* and *Cer1*. These data indicate that BMP signaling in the epiblast induces *Wnt3* and *Wnt3a* expression to maintain WNT signaling in the VE, resulting in downregulation of *Dkk1* to establish the anterior expression domain. Thus, our results suggest that BMP signaling regulates the expression patterns of *Dkk1* for anterior migration of the AVE.

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Introduction

The process by which the anterior–posterior (A–P) body axis is determined during early mouse embryogenesis is of great interest not only because this step is the first body plan of post-implantation mouse embryos, but also because of the complicated nature of the molecular mechanism involved in the development of the anterior visceral endoderm (AVE) (Beddington and Robertson, 1999; Lu et al., 2001; Rossant and Tam, 2004). The AVE is initially formed at the distal end of the visceral endoderm at E5.5 and migrates toward the anterior side of the embryo by E6.0, when the A–P axis becomes morphologically evident (Rivera-Perez et al., 2003; Srinivas et al., 2004). NODAL signaling plays important roles in the formation and migration of the AVE (Brennan et al., 2001; Chen et al., 2006; Ding et al., 1998; Norris et al., 2002; Yamamoto et al., 2004). WNT signaling also affects the migration of the AVE; to enhance or lower the WNT signaling asymmetrically induces unilateral migration of the AVE (Kimura-Yoshida et al., 2005). However, there are many unanswered questions including whether other signaling pathway(s) function in the formation and migration of the AVE and how such pathways relate to NODAL signaling or WNT signaling in the process. For example, Bone Morphogenetic Protein (BMP) signaling is required for the maintenance of *Nodal* expression at E5.5 in the visceral endoderm (VE) (Di-Gregorio et al., 2007). BMP signaling is also required for the expression of *Wnt3* (Ben-Haim et al., 2006), which elicits the majority of the WNT signaling that plays a critical role in migration of the AVE (Kimura-Yoshida et al., 2005).

BMPs comprise a large subgroup within the TGF-beta superfamily. BMP signaling is involved in a variety of functions during developmental process (Kishigami and Mishina, 2005; Zhao, 2003). *Bmpr1a*, which encodes a type I receptor for BMP2 and BMP4, is expressed in all tissues derived from the inner cell mass and trophoblast (Dewulf et al., 1995) and *Bmp4* shows restricted expression in the extraembryonic ectoderm (Lawson et al., 1999). *Bmpr1a* and *Bmp4* constitute the major genes that define BMP signaling in early post-implantation development of mice. Mosaic inactivation of *Bmpr1a* in the epiblast revealed that *Bmpr1a* is required for proper recruitment of epiblast cells during gastrulation (Miura et al., 2006). Deficiency of *Bmpr1a* in the epiblast may also affect VE development (Davis et al., 2004). However, if and how BMP signaling participates in these critical functions during the development of the AVE is not well understood.

In this study, we investigated *Bmpr1a* deficient embryos (*Bmpr-null* embryos) and embryos that lack *Bmpr1a* in an epiblast-specific manner (*Bmpr1a*$^{null/flox}$; *Sox2Cre* embryos) for a potential involvement of BMP signaling in the AVE development. The inactivation of *Bmpr1a* in the epiblast was carried out by recombination of a floxed allele for *Bmpr1a* (Mishina et al., 2002) with *Cre* recombinase

* Corresponding author. Department of Biologic and Materials Sciences, School of Dentistry, University of Michigan, Ann Arbor, MI 48109-1078, USA. Fax: +1 734 647 2110.
E-mail address: mishina@umich.edu (Y. Mishina).

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expressed in *Sox2-Cre* transgenic mice (Hayashi et al., 2002). *Sox2-Cre* drives more efficient *Cre*-mediated recombination from earlier stage of mouse development compared to *Mox2-Cre* (Hayashi et al., 2002). We found that *Bmpr-null* embryos show no migration of the AVE, but *Bmpr1a*$^{null/flox}$; *Sox2Cre* embryos exhibit random migration of the AVE. *Bmpr1a* in the VE is required for *Dkk1* expression in the proximal VE (PxVE). On the other hand, BMP signaling in the epiblast positively regulates the expression of *Wnt3* and *Wnt3a* in the presumptive posterior epiblast, which leads to a downregulation of *Dkk1* in the overlying VE and the migration of AVE cells towards *Dkk1*-expressing cells in the presumptive anterior proximal VE.

Results

The AVE does not migrate in Bmpr-null embryos

Establishment of the A–P axis of the mouse embryo becomes apparent by the migration of the AVE that starts around E5.75 (Rivera-Perez et al., 2003; Srinivas et al., 2004). Although the AVE was formed in *Bmpr-null* embryos as indicated by the expression of *Hex* and *Cer1*, migration of the AVE was not observed in *Bmpr-null* embryos (Figs. 1A–D) ($n=6/6$ for *Hex*, 5/5 for *Cer1*). *Dkk1*, expressed in a circular pattern in the PxVE in control embryos, was not expressed in *Bmpr-null* embryos at E5.5, indicating that *Bmpr1a* is required for its expression (Figs. 1E, F) ($n=5/5$). These data indicate that *Bmpr1a* is required for the migration of the AVE.

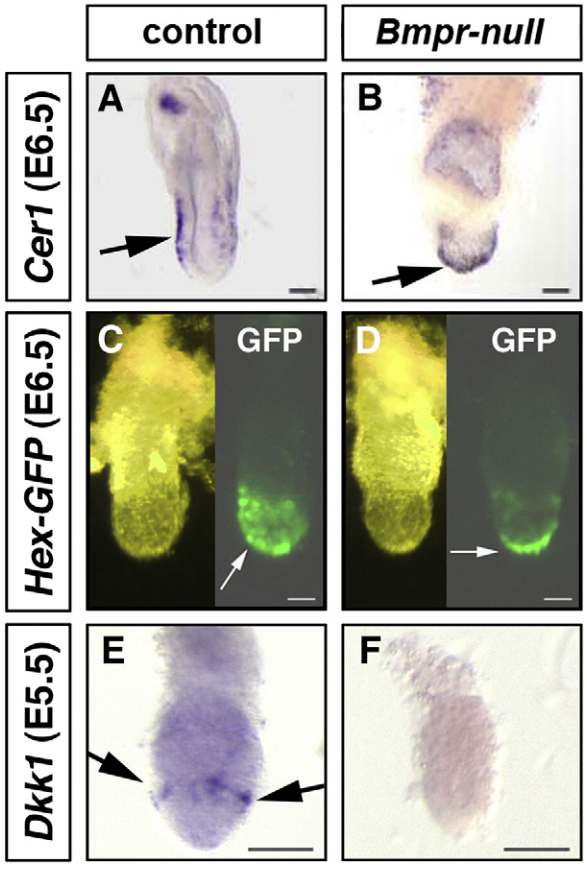

Fig. 1. The AVE does not migrate in *Bmpr-null* embryos. Whole mount in situ analysis for *Cer1* (A, B) or *Dkk1* (E, F) and the expression of *Hex-GFP* (C, D). *Cer1* or *Hex-GFP* was expressed in the AVE of control embryos (A, C arrow). In *Bmpr-null* embryos, *Cer1* and *Hex* expression is localized to the distal tip (B, D, arrow). *Dkk1* is expressed in the PxVE at E5.5 control embryos (E, arrows), but not in *Bmpr-null* embryos (F). Bars = 100 μm.

A–P axis defect is observed in Bmpr1a$^{null/flox}$*; Sox2Cre embryos*

To address the role of BMP signaling in the epiblast for migration of the AVE, we next analyzed *Bmpr1a*$^{null/flox}$; *Sox2Cre* embryos that lack *Bmpr1a* in an epiblast-specific manner (Di-Gregorio et al., 2007). Unlike *Bmpr-null* embryos, *Bmpr1a*$^{null/flox}$; *Sox2Cre* embryos initiate gastrulation to form germ layers (Di-Gregorio et al., 2007) (Figs. 2A–C). Among forty *Bmpr1a*$^{null/flox}$; *Sox2Cre* embryos examined at E8.5, about half of them showed apparent A–P axis evidenced by the expression of *Otx2* and histological analyses (Fig. 2B and data not shown). These embryos were morphologically similar to *Bmpr-MORE* embryos (mosaic inactivation of *Bmpr1a* in the epiblast) that we previously described (Miura et al., 2006). The remaining *Bmpr1a*$^{null/flox}$; *Sox2Cre* embryos had a poorly extended body axis (Fig. 2C). Such variability of the phenotypes may be due to a mixed background of the floxed allele for *Bmpr1a*. These embryos expressed germ layer markers such as *Mox1*, *Foxa2* and *Otx2* (data not shown), suggesting that all three germ layers are formed in *Bmpr1a*$^{null/flox}$; *Sox2Cre* embryos. Expressions of posterior markers, *Brachyury*, *Cripto*, and *Lefty2*, also showed two distinct patterns during gastrulation (Figs. 2D–L). Comparable expression patterns with control embryos were observed in half of the *Bmpr1a*$^{null/flox}$; *Sox2Cre* embryos (Figs. 2E, H, K, 9/17, 8/17, 4/8, respectively). However, in the rest of the *Bmpr1a*$^{null/flox}$; *Sox2Cre* embryos, posterior markers that are normally detected at the primitive streak were expressed at the proximal potion of the epiblast (Figs. 2F, I, L, 8/17, 9/17, 4/8, respectively). Di-Gregorio et al. reported downregulation of *Cripto* in the *Bmpr1a*$^{null/flox}$; *Sox2Cre* embryos (3/9) (Di-Gregorio et al., 2007), but all of the mutant embryos examined here showed comparable levels of expression despite the differences of expression domains Figs. 2H–I, 17/17). This may be due to the differences of genetic background, because we maintained the floxed allele for *Bmpr1a* in a mixed background. These results indicate that there was an A–P axis defect of the epiblast development in half of the *Bmpr1a*$^{null/flox}$; *Sox2Cre* embryos during gastrulation, leading to poor extension of the body axis by E8.5 (Figs. 2C).

Next, we examined expressions of AVE markers such as *Cer1*, *Hex* at E6.0, since the AVE normally migrates to the anterior side of the embryo by this stage, as well as *Dkk1* expression. Expression patterns of *Cer1* and *Hex* showed that the AVE does not migrate in half of the *Bmpr1a*$^{null/flox}$; *Sox2Cre* embryos by E6.0 (3/5 for *Cer1* and 7/15 for *Hex*) (Figs. 3B, F). *Dkk1* was expressed in the anterior PxVE of control embryos at E6.0 (Fig. 3K). In *Bmpr1a*$^{null/flox}$; *Sox2Cre* embryos, *Dkk1* was expressed circumferentially in the PxVE (3/6) (Fig. 3L). During E5.5 and 5.75, the *Dkk1* expression pattern shows a dramatic change in normal development (Kimura-Yoshida et al., 2005). In control embryos, *Dkk1* expression was observed in a circular pattern in the PxVE at E5.5 (3/3) (Fig. 3G). However, *Dkk1* was strongly downregulated from most of the PxVE, leaving a small expression domain at the most anterior part of the PxVE at E5.75 (7/7) (Fig. 3I). In contrast, *Dkk1* was still expressed circularly in the PxVE in *Bmpr1a*$^{null/flox}$; *Sox2Cre* embryos (2/4 at E5.5, Fig. 3H, 4/8 at E5.75, Fig. 3J), suggesting that downregulation of the *Dkk1* did not occur at E5.75.

We further compared the changes in expression domains of *Dkk1* along with migration of the AVE. At E5.65, 3 embryos out of 4 showed anteriorly shifted expression of *Dkk1* (Fig. 3M). In these embryos, the AVE marked by *Cer1* was localized at the distal tip of the embryos (Fig. 3M, 2/3). At E5.75, the embryos showed anterior movement of the AVE (3/3) and such embryos also showed anteriorly shifted expression of *Dkk1* (Fig. 3N). At E6.25 and E6.5, *Dkk1* was expressed as a horseshoe shape in a domain adjacent to upper part of the AVE of control embryos (Figs. 3O–R). These results indicate that anterior shift of the expression domains of *Dkk1* preceded migration of the AVE.

Expansion of the AVE caused by ectopic migration of the AVE cells in Bmpr1a$^{null/flox}$*; Sox2Cre embryos*

By E6.5, the AVE further migrated anteriorly in control embryos (Figs. 4A, C, E, H). However, *Cer1*, *Hesx1*, *Lefty1* and *Hex* expression