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Bmp4 and Noggin expression during early thymus and parathyroid organogenesis

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Abstract

The thymus and parathyroids originate from the third pharyngeal pouches, which form as endodermal outpocketings in the pharyngeal region beginning on embryonic day 9 (E9.0) of mouse development. Using organ-specific markers, we have previously shown that thymus and parathyroid-specific organ domains are established within the primordium prior to formation of the organs proper: *Gcm2* expression defines the prospective parathyroid cells in the dorsal pouch from E9.5, while *Foxn1* is expressed in the thymus domain from E11.25. Bmp (bone morphogenetic protein) signaling has been implicated in thymic epithelial cell differentiation and thymus organogenesis. In the present study, we report expression patterns of *Bmp4* and *Noggin*, a Bmp4 antagonist, in the third pharyngeal pouch using two lacZ transgenic mouse strains. Results from this gene expression study revealed localization of *Bmp4* expression to the ventral region of the third pharyngeal pouch endoderm at E10.5 and E11.5, in those cells that will express Foxn1 and form the thymus. Conversely, the expression of *Noggin* was confined to the dorsal region of the pouch and primordium at these stages, and thus appeared to be co-expressed with *Gcm2* in the parathyroid domain. This represents the first detailed study of *Bmp4* and *Noggin* expression during the early stages of thymus and parathyroid organogenesis.

Keywords: Bmp4; Noggin; Thymus; Parathyroid; Pharyngeal pouch; Endoderm; Organogenesis; Foxn1; Gcm2

1. Results and discussion

The pharyngeal pouches are paired structures that arise as outpocketings of the lateral surfaces of the foregut endoderm during embryogenesis. The third pharyngeal pouches form on embryonic day 9–10 (E9.0–E10.0) of mouse development and give rise to the thymus and parathyroids. Cells in the ventral and posterior domain of the primordium will differentiate into thymic epithelial cells, and can be identified after E11 by the expression of *Foxn1* (Gordon et al., 2001), a transcription factor required cell-autonomously for thymic epithelial cell differentiation (Blackburn et al., 1996). The dorsal and anterior cells express *Gcm2*, one of two mammalian orthologs of the *Drosophila glial cells missing* transcription factor (Kim et al., 1998), and will form the parathyroids (Gordon et al., 2001). The molecular mechanisms by which this organ-specific identity is established within the third pharyngeal pouch and common thymus-parathyroid primordium are not understood.

Bone morphogenetic proteins (BMPs) are members of the TGF-β superfamily of intercellular signaling proteins, and their involvement in the patterning and cellular fate determination of many organs and systems during the development of many organisms is well established (Hogan, 1996). We have shown via whole mount in situ hybridization that *Bmp4* is expressed in the ventral/posterior prospective thymus domain of the third pharyngeal pouch at E10.5 (Moore-Scott and Manley, 2005), and this

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was confirmed in a more recent study (Bleul and Boehm, 2005). We demonstrated that this *Bmp4* expression was opposed by a dorsal domain of sonic hedgehog (*Shh*) expression, and that in the absence of Shh, the domain of *Bmp4* expression extended dorsally (Moore-Scott and Manley, 2005). Interestingly, *Foxn1* expression at E11.5 also expanded throughout the entire primordium, while *Gcm2* expression was absent, suggesting that a Bmp signal may be responsible for thymus identity. This was consistent with previous studies defining Bmp4 as a positive regulator of *Foxn1* in thymic epithelial cells in vitro (Tsai et al., 2003). However a detailed study of *Bmp4* expression during early thymus and parathyroid organogenesis has not yet been performed.

Bmp signaling is tightly regulated by a number of extracellular inhibitors, such as noggin, chordin, follistatin and twisted gastrulation, all of which function by directly binding BMPs to prevent the activation of cell surface receptors (Chang et al., 2001; Piccolo et al., 1996; Yamashita et al., 1995; Zimmerman et al., 1996). Roles for noggin, chordin and twisted gastrulation have been proposed in later stages of mouse thymus development (Graf et al., 2002; Hager-Theodorides et al., 2002; Nosaka et al., 2003; Scott et al., 2000). Chordin has also been shown to be required during pharyngeal region development: *Chordin* is expressed in the endoderm of the dorsal pharynx at E9.0, and a loss of gene function results in absence of the caudal pharyngeal arches and pouches (Bachiller et al., 2003). In addition, *Noggin* expression has been reported in the pharyngeal arch mesenchyme at E9.5 (Stottmann et al., 2001). These reports suggest the importance of restricting Bmp signaling during pharyngeal development. In the current studies, we have expanded on our previous observations of *Bmp4* expression in the third pharyngeal pouch, focusing on the dynamic expression of *Bmp4* and the Bmp4 antagonist *Noggin*.

We used $Bmp4^{lacZ}$ and $Noggin^{lacZ}$ transgenic mouse strains (Lawson et al., 1999; McMahon et al., 1998) to perform a detailed study of *Bmp4* and *Noggin* expression during the early stages of thymus and parathyroid organogenesis. At E9.5 the third pharyngeal pouches have just formed and are essentially small buds on the lateral surfaces of the pharynx, consisting of a single epithelial cell layer surrounding a central lumen that is continuous with the pharynx. At this stage, neither *Bmp4* nor *Noggin* was expressed in the third pharyngeal pouch endoderm (Figs. 1A and B and Figs. 2A and B). There was, however, some *Bmp4* expression in the ventral pharynx, just medial to the entrance of the third pouch (Fig. 1A), and a few *Noggin*-expressing cells were detected in the second pouch (Fig. 1B). *Bmp4* and *Noggin* were both expressed in the pharyngeal arch mesenchyme at E9.5, but in different patterns. As previously reported (Stottmann et al., 2001), *Noggin* was expressed throughout the mesenchyme of the third pharyngeal arch, including those cells immediately adjacent to the early third pharyngeal pouch (Fig. 1B). In contrast, *Bmp4* expression in the third arch was restricted to a few mesenchymal cells in the core, possibly corresponding to mesoderm, but never in those cells closest to the endoderm (Fig. 1A). *Bmp4* expression was also observed in the overlying ectoderm of the third pharyngeal arch and cleft, and in the mesenchyme caudal to the fourth arch and immediately adjacent to the surface ectoderm (Fig. 1A). At this stage, *Noggin* expression was absent from all surface ectoderm in the pharyngeal region (Fig. 1B).

By E10.5, both *Bmp4* and *Noggin* were expressed in cells of the third pharyngeal pouch endoderm (Figs. 2C and D). At this stage, the most lateral part of the third pouch endoderm contacts the overlying ectoderm of the third pharyngeal cleft (Gordon et al., 2004). This contact is transient; the two cell layers separate from each other at around E11.5, and we have demonstrated via lineage tracing that the ectodermal cells do not physically contribute to the thymic epithelium (Gordon et al., 2004). At E10.5, *Bmp4* expression within the third pharyngeal pouch endoderm was present throughout the ventral and posterior portion, consistent with our previous observations using whole-mount in situ hybridization (Moore-Scott and Manley, 2005). Coronal sections at E10.5 revealed a difference in the distribution of *Bmp4* expression between ventral and dorsal aspects of the third pouch (Figs. 1C and D): expression was present throughout the posterior aspect of the pouch in more ventral sections (Fig. 1C), while in more dorsal sections only those cells in close contact with the third pharyngeal cleft ectoderm expressed *Bmp4* (Fig. 1D). The posterior region of the fourth pharyngeal pouch also expressed *Bmp4* at E10.5 (Fig. 1D). Sections cut in a transverse plane confirmed the ventral and posterior localization of *Bmp4* within the third pharyngeal pouch (Figs. 1E and F), and also showed expression throughout the ventral pharynx (Fig. 1E). Sagittal sections further confirmed localization of *Bmp4* expression to a small proportion of cells at the ventral aspect of the third pouch (Fig. 1K). The third pharyngeal cleft at E10.5 expressed higher levels of Bmp4 than the endoderm (Fig. 1D). We also observed Bmp4 expression in presumptive neural crest-derived mesenchyme cells immediately adjacent to the *Bmp4*-expressing endodermal cells (Figs. 1E and K). This supports recent evidence for Bmp-mediated epithelial-mesenchymal interactions in early thymus development (Bleul and Boehm, 2005), and is reminiscent of events seen during tooth development (Vainio et al., 1993).

At E10.5, mesenchymal expression of *Noggin* was suddenly and dramatically down regulated (Figs. 1G and H). Thus, unlike *Bmp4*, at this stage *Noggin* expression was excluded from the surrounding mesenchyme and from the third pharyngeal cleft ectoderm (Figs. 1G–J and L), and was instead confined to cells in the most anterior and dorsal region of the third pouch endoderm. Coronal sections (Figs. 1G and H) showed differences in the anterior and posterior regions of the pouch, as well as ventral and dorsal differences. A section through the ventral portion of the third pharyngeal pouch contained no *Noggin*-expressing