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BMP4 signaling directs primitive endoderm-derived XEN cells to an extraembryonic visceral endoderm identity

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ABSTRACT

The visceral endoderm (VE) is an epithelial tissue in the early postimplantation mouse embryo that encapsulates the pluripotent epiblast distally and the extraembryonic ectoderm proximally. In addition to facilitating nutrient exchange before the establishment of a circulation, the VE is critical for patterning the epiblast. Since VE is derived from the primitive endoderm (PrE) of the blastocyst, and PrE-derived eXtraembryonic ENdoderm (XEN) cells can be propagated *in vitro*, XEN cells should provide an important tool for identifying factors that direct VE differentiation. In this study, we demonstrated that BMP4 signaling induces the formation of a polarized epithelium in XEN cells. This morphological transition was reversible, and was associated with the acquisition of a molecular signature comparable to extraembryonic (ex) VE. Resembling exVE which will form the endoderm of the visceral yolk sac, BMP4-treated XEN cells regulated hematopoiesis by stimulating the expansion of primitive erythroid progenitors. We also observed that LIF exerted an antagonistic effect on BMP4-induced XEN cell differentiation, thereby impacting the extrinsic conditions used for the isolation and maintenance of XEN cells in an undifferentiated state. Taken together, our data suggest that XEN cells can be differentiated towards an exVE identity upon BMP4 stimulation and therefore represent a valuable tool for investigating PrE lineage differentiation.

Introduction

The patterning of the early mouse embryo relies on reciprocal signaling interactions between embryonic and extraembryonic tissues (reviewed in (Arnold and Robertson, 2009; Rossant and Tam, 2009)). The two extraembryonic tissues, trophectoderm (TE) and primitive endoderm (PrE), are specified and spatially segregated away from the pluripotent epiblast (EPI) prior to embryo implantation into the maternal uterus (reviewed in (Cockburn and Rossant, 2010; Zernicka-Goetz et al., 2009)). TE is the first extraembryonic lineage established and forms an epithelial layer surrounding the inner cell mass (ICM) of the blastocyst stage embryo. TE will form the trophoblast giant cells and the extraembryonic ectoderm (ExE), essential for the establishment of a fetal–maternal connection. The PrE is one of the two cell populations specified within the ICM, the other being the EPI. PrE and EPI cells emerge initially in an apparent salt-and-pepper fashion but, by the end of the preimplantation period, are spatially segregated, with the PrE forming a single cell layer on the surface of the ICM in contact with the blastocoel cavity (Chazaud et al., 2006; Meilhac et al., 2009; Plusa et al., 2008). Thus, at the time of blastocyst implantation, the pluripotent EPI is encapsulated by the TE and the PrE (Fig. 1A).

After implantation, the PrE differentiates into the parietal endoderm (PE) and the visceral endoderm (VE). The PE, which lies adjacent to trophoblast giant cells, will form the endoderm of the parietal yolk sac, while the majority of the VE will come to lie adjacent to extraembryonic mesoderm where it will form the visceral yolk sac, the site of primitive hematopoiesis and vasculogenesis (Fig. 1A). Cell transplantation experiments have revealed some

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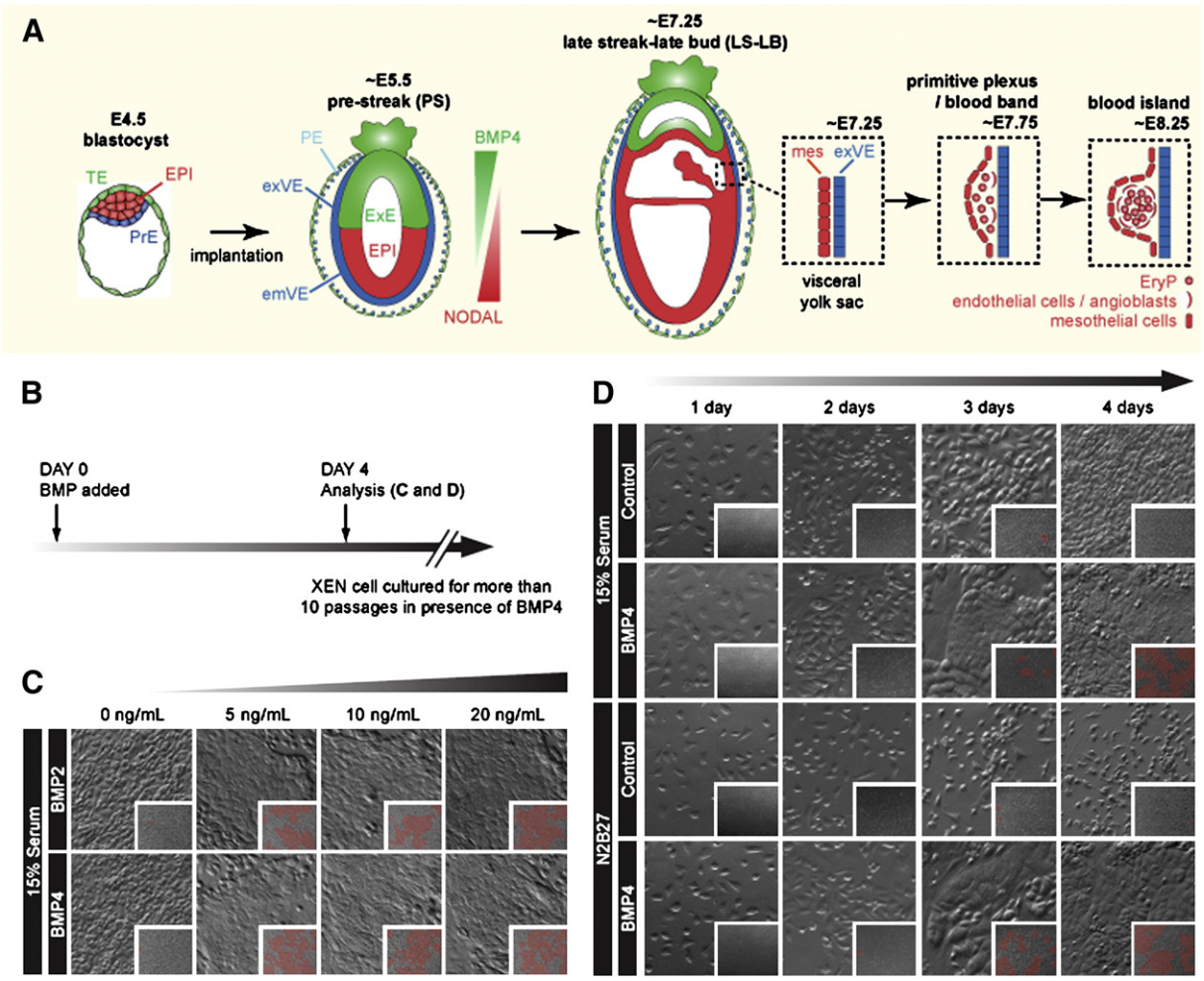

Fig. 1. BMP treatment alters XEN cell morphology. (A) Schematic representation of periimplantation (E4.5) and early postimplantation (E5.5 and E7.25) stage mouse embryos. High magnification insets on the region where the exVE and extraembryonic mesoderm are apposed, representing the site of blood island formation (Ferkowicz and Yoder, 2005; Li et al., 2005). Red, EPI and its derivatives; green, TE and TE derivatives; blue, PrE and PrE derivatives. (B) Diagramatic representation of the timeline of BMP treatment. (C) XEN cell morphology after addition of BMP2 and BMP4 for 4 days at concentrations ranging from 5 to 20 ng/mL in serum culture conditions. (D) Kinetics of the morphological changes upon addition of 10 ng/mL of BMP4 in serum and serum-free (N2B27) conditions. (C-D) High magnification zooms of low magnification images (inset) acquired with a 10× magnification. In the insets, areas of epithelial cell colonies are highlighted in red.

degree of lability between these two PrE derivatives, as VE cells from early postimplantation embryos (around embryonic day (E) 5.5 — Fig. 1A) transplanted to recipient blastocysts were able to contribute to both PE and VE (Gardner, 1982; Hogan and Tilly, 1981). Even so, these cell types can be distinguished by their morphology and their distinct biological properties. PE cells are characterized by their dispersed distribution and migratory behavior along the mural TE. They secrete the extracellular matrix proteins that comprise Reichert's membrane. By contrast, the VE forms an epithelial monolayer that encapsulates the ExE proximally (where it is also referred as exVE) and the EPI distally (where it is also referred to as emVE).

The VE is required for embryo survival and patterning. It functions as the primary site of gas, nutrient and waste exchange prior to the establishment of a circulation. As a polarized epithelium it possesses the necessary intracellular machinery for directional absorption and transport of compounds (reviewed in (Bielinska et al., 1999)). At periimplantation stages, the VE plays a role in epiblast cell survival as well as in the formation of the proamniotic cavity (Coucouvanis and Martin, 1995, 1999). More recently, it has been shown that specialized regions within the VE are required for embryonic axis formation and epiblast patterning. For example at E5.5, a population of molecularly and morphologically distinct emVE cells is evident at the distal tip of the embryo (Rivera-Perez et al., 2003). This population, referred to as the distal VE (DVE), expresses transcription factors such as *Lhx1* and *Hhex*, and secretes antagonists of Wnt and Nodal signaling including CER1, DKK1 and LEFTY1 (reviewed in (Pfister et al., 2007)). Live imaging studies have revealed that DVE cells actively and collectively migrate proximally within the plane of the emVE epithelium towards the embryonic-extraembryonic junction (Migeotte et al., 2010; Srinivas et al., 2004), as they will converge with a second population the anterior VE (AVE) (Takaoka et al., 2011; Thomas and Beddington, 1996). This translocation of DVE/AVE cells effectively converts the proximal-distal axis of the periimplantation embryo into an anterior-posterior axis and results in the correct positioning of the primitive streak, defining the posterior side of the embryo concomitant with the start of gastrulation.

Gastrulation cell movements will give rise to the visceral yolk sac, where exVE becomes apposed to extraembryonic mesoderm (Fig. 1A). This is the site of specification of hematopoietic and endothelial progenitors, which are organized into blood islands (reviewed in (Baron, 2005; Fraser and Baron, 2009; Medvinsky et al., 2011)). Instructive signals emanating from exVE are essential in this process, and exVE can reprogram anterior epiblast into posterior fates (Belaoussoff et al., 1998). Candidate exVE secretory factors include Vascular Endothelial Growth Factor (VEGF) and Indian Hedgehog (IHH) (Dyer et al., 2001; Pierre et al., 2009).