Polarized expression of bone morphogenetic protein-4 in the human aorta-gonad-mesonephros region

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In the mammal, definitive hematopoietic stem cells (HSCs) are first derived from mesodermal cells within a region of the embryonic para-aortic splanchnopleura known as the aorta-gonad-mesonephros (AGM). Within this region, HSCs are thought to arise from hemangioblast precursors located in the ventral wall of the dorsal aorta. However, the factors that regulate HSC development in vivo are still largely unknown. Bone morphogenetic protein (BMP)-4, a member of the transforming growth factor beta (TGF-β) superfamily of growth factors, is a potent ventralizing factor and has been implicated in the commitment of embryonic mesodermal cells to a hematopoietic fate in a number of systems. In the human AGM, we find that BMP-4 is expressed at high levels, and with striking polarity, in a region of densely packed cells underlying intra-aortic hematopoietic clusters. In contrast, TGF-β1 is expressed predominantly by hematopoietic cells within the clusters. These findings implicate both BMP-4 and TGF-β1 in the initiation and regulation of hematopoiesis in the human AGM. Furthermore, the distribution of BMP-4 expression is highly suggestive of a direct role in the specification of human hematopoietic cells from embryonic mesoderm in vivo. (Blood. 2000;96:1591-1593)

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Introduction

The major source of definitive murine HSCs has been mapped to the embryonic AGM, at a time when clusters of hematopoietic cells are found adhering to the ventral wall of the dorsal aorta.1,2 The appearance of clusters is restricted to between 9.5 to 11.5 days post coitum in the mouse and 30 to 37 days gestation in the human.3,4 These cell clusters express a number of molecules in common with endothelial cells lining the wall of the dorsal aorta, including the membrane glycoprotein CD34, the transcription factors SCL and GATA-2, and the growth factors receptor c-Kit and FLK-1.5,6 The shared expression patterns and the close physical association between the HSC cluster and the endothelium has led to the suggestion that both lineages arise from a common hemangioblast precursor located within or underlying the wall of the dorsal aorta. In addition, the marked asymmetry of this process suggests that cluster formation is not a stochastic event, but one that is determined by microenvironmental signals localized to the ventral aortic wall.

Thus far, the factors that regulate hemangioblast differentiation in vivo remain unknown although analysis of hematopoietic development in lower vertebrates suggests that members of the TGF-β superfamily of secreted polypeptide growth factors, including the bone morphogenetic proteins (BMP) play a critical role. During embryogenesis, BMPs are thought to specify a variety of cell fates dependent on concentration gradients, and expression of antagonistic factors such as activin and noggin.7 One family member, BMP-4, is known to induce ventral mesoderm, the tissue that will eventually give rise to the hematopoietic system. Evidence from studies using Xenopus laevis suggests that BMP-4 is also involved in the commitment of mesodermal cells to the blood lineage.8 In Xenopus, HSCs arise in the embryonic ventral mesoderm, which contains the dorsal lateral plate, a region analogous to the mammalian AGM. Ectoderm-derived animal cap cells, which do not normally give rise to blood cells, can be induced to do so in vivo after treatment with ectopic BMP-4. After mesoderm has formed, expression of a dominant negative BMP-4 receptor inhibits normal hematopoietic development, and mutant embryos lacking BMP-4 expression also fail to develop hematopoietic tissue. These data support a role for BMP-4 not only in mesoderm formation but also in the conversion of mesoderm to blood, possibly by regulating the temporal and spatial expression of necessary transcription factors within the hemangioblast population.

The involvement of BMP-4 in the initiation of mammalian hematopoiesis has been less easy to study. For example, mice lacking BMP-4 seldom develop beyond the egg cylinder stage, preceding the appearance of blood cells because of a critical requirement during gastrulation. However, in rare cases where homozygous mutants have developed to later stages, there is a noticeable paucity of blood cells circulating in the vasculature.10 On the other hand, the addition of BMP-4 to murine embryonic stem (ES) cells results in the acquisition of mesodermal markers and development of myeloid hematopoietic precursors. Interestingly, the dose of BMP-4 required for mesoderm formation from ES cells is considerably lower than that required for maximal hematopoietic output, suggesting that BMP-4 is sufficient to initiate hematopoiesis from existing mesodermal tissues, but in a concentration dependent manner.11

Unlike BMPs, TGF-β1 itself is not thought to be directly associated with dorsoventral patterning, but is an important regulator of cell proliferation and differentiation in a variety of tissues during embryogenesis. Mice lacking TGF-β1 mainly die in utero.
between 9.5 to 11.5 days post coitum because of defects in yolk sac hematopoiesis and vasculogenesis. In addition, TGF-β1 has been shown to inhibit the initial proliferation and differentiation of murine long-term repopulating HSCs and primitive human long-term culture-initiating cells. Early human hematopoietic progenitors themselves secrete TGF-β1, and it has been suggested that this autocrine production may be important in the negative regulation of cell cycling.

Study design

Human tissue collection and processing

Human embryos and yolk sacs were obtained from the MRC-funded Human Embryo Bank maintained at the Institute of Child Health. The embryos were staged and processed as previously described.

Immunohistochemistry

Paraffin-embedded tissue sections through human embryos at 28, 34, and 38 days gestation and human yolk sacs were pretreated and antibody-binding visualized as described previously. Transverse sections were incubated with mouse monoclonal antibodies raised against human BMP-4 (clone 3H2; Novocastra Laboratories, UK) and TGFβ1 (clone TGFβ17; Novocastra Laboratories, UK). For TGFβ1, a high-temperature antigen-unmasking technique was used. Sections were incubated with primary antibody for 60 minutes at room temperature. Results shown are representative.

Results and discussion

Immunohistochemical analysis of the human embryonic AGM reveals clusters of hematopoietic cells associated with the ventral wall of the dorsal aorta. These cells express the membrane glycoprotein CD34 and the hematopoietic-specific marker CD45. We have also previously reported the presence of a morphologically distinct region resembling a stromal layer in the mesenchyme directly beneath the ventral wall of the dorsal aorta in the AGM that is associated with intra-aortic clusters. This consists of between 5 and 7 layers of densely packed cells in the 34-day human embryo (Figure 1A and B) or 3 to 4 layers in the mouse at 10.5 days post coitum, and expresses high levels of the extracellular matrix protein tenascin-C (data not shown). In view of their emerging importance in the development of hematopoiesis, we investigated whether expression of members of the TGF-β family is localized to this region. In the 34-day human embryo, BMP-4 was expressed in a gradient across the dorsal-ventral axis with many highly positive cells distributed throughout the ventral mesoderm, including transected descending neuronal tracts (Figure 1A). Within the AGM region, compared with the surrounding tissues and adjacent cardinal vessels, expression of BMP-4 was polarized to the ventral wall of the dorsal aorta, specifically in the region of the “stromal” layer and underlying the intra-aortic clusters (Figure 1B). At 28 days gestation, no distinct dorsal-ventral gradient of expression was apparent, neuronal tracts were absent and BMP-4 expression within the ventral mesoderm was generally low, with few strongly positive cells (Figure 1C). However, at this earlier developmental stage, emerging clusters of hematopoietic cells were also found adhering to the ventral wall of the dorsal aorta and BMP-4 expression was strikingly restricted to the “stromal” layer, which consisted of only 2 or 3 layers of cells at this age, underlying these clusters (Figure 1D).

Outside the AGM, at more caudal and rostral locations, the level of BMP-4 expression was comparatively low and no hematopoietic clusters were observed (Figure 1E and data not shown). Similarly, in older (38-day) embryos, after intra-aortic clusters have disappeared, BMP-4 expression around the dorsal aorta was no longer polarized (Figure 1F). Interestingly, the analysis of human embryonic yolk sac also revealed strong BMP-4 expression in cells specifically associated with blood islands (Figure 1G).

We also investigated the expression pattern of TGF-β1. In contrast to BMP-4, the expression of TGF-β1 was uniformly low.
around the dorsal aorta, but detectable at higher levels in the cytoplasm of hematopoietic cells within the intra-aortic clusters (Figure 1H). No positive staining was observed in controls using normal serum or secondary antibody only.

In summary, we find that BMP-4 is expressed at high levels in a cell-density region underlying the ventral wall of the dorsal aorta at the time of human AGM hematopoiesis. Taken together with evidence from other developmental systems, this may be a critical requirement for the initiation of a hematopoietic differentiation program from mesodermal precursors. The factors that regulate expression of BMP-4, and downstream signaling events that determine this process are at present unclear, but may involve tightly regulated expression (spatial and temporal) of transcription factors such as Tel-1/SCL, Lmo2, GATA-2, and AML1/Cbfa2, a member of the family of core-binding transcription factors.\(^\text{16-20}\) For example, in a parallel system, the induction of core-binding factor AML3 by BMP family members has been shown to be tightly linked to the process of osteoblastic differentiation.\(^\text{21,22}\) AML1 is now known to be required for definitive hematopoiesis\(^\text{23,24}\) and is transiently expressed in cells in the ventral wall of the dorsal aorta in the murine AGM coincident with the appearance of hematopoietic clusters.\(^\text{25}\) In the embryonic AGM, BMP-4 may therefore directly influence the conversion of hemangioblasts to committed HSCs through induction of AML1. The contrasting expression patterns of BMP-4 and TGF-β1 in the embryonic AGM are consistent with a role for BMP-4 in the initiation of HSC development from mesodermal precursors, and for TGF-β1, possibly together with BMP-4, in the regulation of hematopoietic cell fate.

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References

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