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Extravascular endothelial and hematopoietic islands form through multiple pathways in midgestation mouse embryos

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ABSTRACT

The *de novo* generation of hematopoietic cells occurs during midgestation when a population of endothelial cells called hemogenic endothelium transitions into hematopoietic progenitors and stem cells. In mammalian embryos, the newly formed hematopoietic cells form clusters in the lumens of the major arteries in the embryo proper and in the vascular plexus of the yolk sac. Small clusters of hematopoietic cells that are independent of the vasculature (referred to here as extravascular islands) were shown to form in the mesentery during vascular remodeling of the vitelline artery. Using three-dimensional imaging of whole mouse embryos we demonstrate that extravascular budding of hematopoietic clusters is a more widespread phenomenon that occurs from the vitelline and the umbilical arteries both proximal to the embryo proper and distal in the extraembryonic yolk sac and placenta. Furthermore, we show that there are several mechanisms by which hematopoietic clusters leave the arteries, including vascular remodeling and extrusion. Lastly, we provide static images suggesting that extravascular islands contribute to the formation of new blood vessels. Thus, extravascular islands may represent a novel mechanism of vasculogenesis whereby established vessels contribute endothelial and hematopoietic cells to developing vascular beds.

1. Introduction

During embryogenesis, definitive hematopoietic progenitors and stem cells (HSPCs) are derived *de novo* from a transient population of endothelial cells called hemogenic endothelium (HE). HE is located in the large diameter arteries including the vitelline artery, umbilical artery and dorsal aorta, and is also found in the heart, yolk sac and the head (Li et al., 2012; Nakano et al., 2013; Frame et al., 2015). HE cells are the precursors to hematopoietic cells, and as such transition into the full repertoire of definitive hematopoietic cells including erythro-myeloid progenitors, lymphoid progenitors, and hematopoietic stem cells (HSCs) during a process termed the endothelial to hematopoietic transition (EHT). Live-imaging studies demonstrated that during the EHT, HE cells bend away from the endothelial layer eventually releasing contact with neighboring endothelial cells and acquiring hematopoietic morphology in a cell-division independent process (Kissa and Herbomel, 2010; Lam et al., 2010; Boisset et al., 2010). The EHT is dependent upon expression of the transcription factor Runx1 (Lancrin et al., 2009; Chen et al., 2009; Boisset et al., 2010; North et al., 1999; Kissa and Herbomel, 2010; Yokomizo et al., 2001). Runx1 expression initiates a hematopoietic transcriptional program and inhibits an endothelial program in HE allowing the EHT to progress (Lancrin et al., 2012; Lichtinger et al., 2012; Hoogenkamp et al., 2009). Runx1 is widely considered the master regulator of hematopoiesis and is the most reliable marker of HE identity.

After the EHT, the newly formed hematopoietic cells take different paths in different species. In zebrafish, hematopoietic cells bud away from the lumen of the artery into the sub-aortic space, and enter the circulation via intravasation through the axial vein (Murayama et al., 2006; Kissa et al., 2008). In chick embryos the EHT occurs both into the aortic lumen and also out of the aorta into the subaortic mesenchyme (Jaffredo et al., 2000, 1998). In mammalian embryos the newly formed hematopoietic cells bud into the lumen of the artery where they remain briefly attached to the endothelium forming clusters of hematopoietic cells (Yokomizo and Dzierzak, 2010; Garcia-Porrero et al., 1995). Hundreds of hematopoietic clusters line the major arteries of the midgestation

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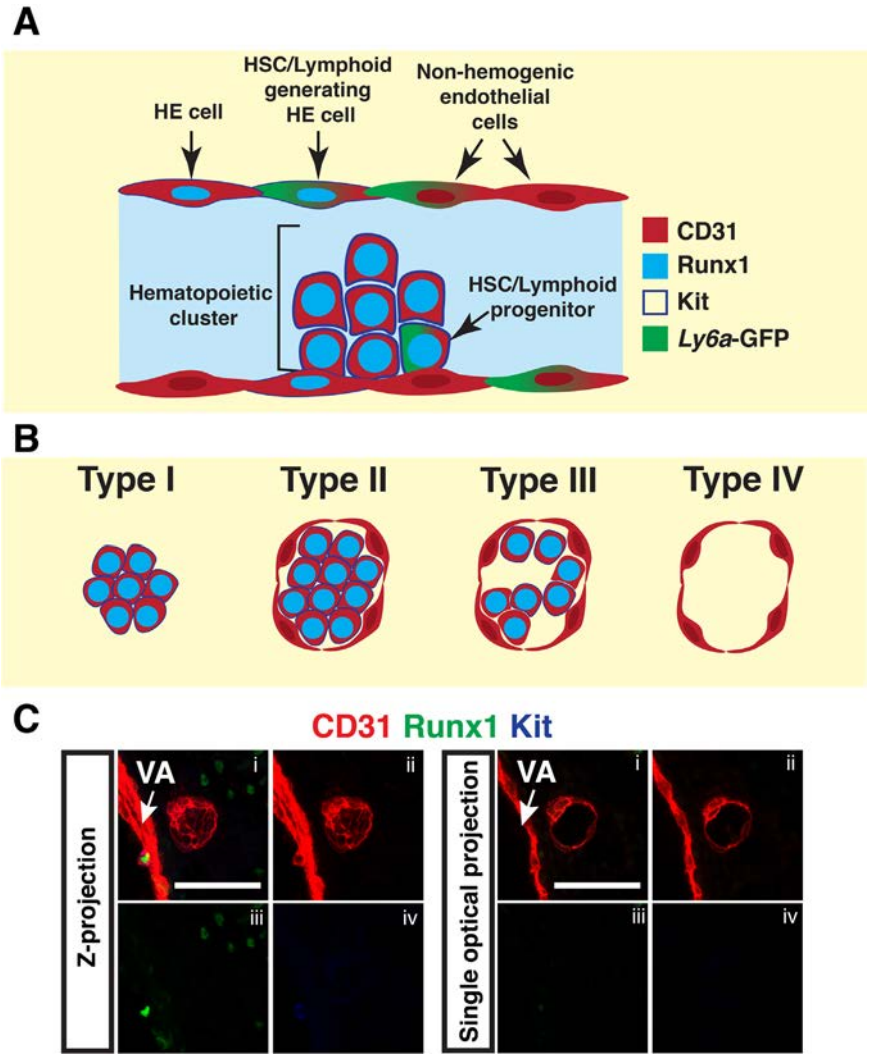

Fig. 1. Four types of extravascular islands. (A) Schematic illustrating the cell types identified by the combination of markers used in the analysis. (B) The four types of extravascular islands. Type I consists of tightly arranged hematopoietic cells with no endothelial component. Type II islands contain tightly arranged hematopoietic cells wrapped in a single layer of endothelial cells. Type III has loosely arranged hematopoietic cells wrapped in an endothelial layer. Type IV consists of a sphere of endothelial cells that are not associated with hematopoietic cells. (C) Z-projection of a type IV extravascular island that has been immunostained for CD31 (i,ii), Runx1 (i,iii) and Kit (i,iv). Panel on the right is a single optical projection illustrating the lack of Runx1^{+} Kit^{+} hematopoietic cells within a type IV extravascular island. Z-interval=2 μm and scale bar=50 μm.

mouse embryo and dozens can be found within the vascular plexus of the yolk sac (Yokomizo and Dzierzak, 2010; Frame et al., 2015). Hematopoietic clusters are heterogeneous, as individual cells within a single cluster can differ in gene expression and function (Yokomizo and Dzierzak, 2010). For example, the *Ly6a*-GFP transgene (*Ly6a* encodes Sca-1) was shown to mark a subset of hematopoietic cluster cells that are enriched for HSCs and lymphoid progenitors (Li et al., 2014; De Bruijn et al., 2002).

Clusters of hematopoietic cells wrapped in endothelial cells were observed in the mesentery of chick embryos one hundred years ago (Miller, 1913). These structures became know as mesenteric blood islands owing to their separation from the cardiovascular system. Mesenteric blood islands were observed in the vicinity of the aortic arches and along the aorta as far as the superior mesenteric artery (the structure that supersedes the vitelline artery) in 100–110 h chick embryos (Miller, 1913). In the 1990s mesenteric blood islands were also observed in mouse embryos between embryonic day (E) 9.5 and E11.5 (Garcia-Porrero et al., 1998; Garcia-Porrero et al., 1995). At the time, the origin of mesenteric blood islands was unknown, but immunohistochemistry of E11 mouse sections illustrated that mesenteric blood islands expressed the endothelial/hematopoietic markers CD31, CD34 and vWf, similar to intra-arterial clusters (Garcia-Porrero et al., 1998). Further histological analysis of sectioned mouse embryos revealed three types of mesenteric blood islands: type I consisting of tightly arranged, highly basophilic, electron-dense cells with no clear endothelial component, type II characterized by undifferentiated hemocytoblasts that are tightly wrapped by endothelial cells, and type III consisting of erythroblasts and hemocytoblasts that are loosely arranged and circumscribed by a single layer of endothelial cells (Garcia-Porrero et al., 1995). The vitelline artery was later identified as the source of mesenteric blood islands (Zovein et al., 2010). During midgestation the vitelline artery undergoes extensive remodeling, and during this process clusters of hematopoietic cells leave the artery and migrate through the mesentery as aggregates of endothelial and hematopoietic cells (Zovein et al., 2010). However, the extravascular emergence of hematopoietic clusters was called into question when studies using three-dimensional imaging of whole mouse embryos only found hematopoietic clusters within arterial lumens (Yokomizo et al., 2011).

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