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Review article

The origin of the first blood cell

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Abstract

In mouse development, the first blood cell activity appears in the yolk sac at E7.5 (embryonic day 7.5). However, this blood cell activity (primitive hematopoiesis) is restricted to red blood cells (primitive erythrocytes). The pluripotent hematopoietic stem cells which are able to differentiate into all kinds of peripheral blood cell (including lymphocytes) are initially identified in the AGM (aorta-gonad-mesonephros) region at E10.5 (definitive hematopoiesis). *In vitro* studies suggest the presence of hemangioblasts, which can differentiate into both blood and endothelial cells. However, until recently, direct evidence of the presence of these hemangioblasts *in vivo* was not available, and the precise mechanism of the differentiation of hematopoietic cells from hemangioblasts was totally unknown. Recent progress in this field of study indicates that the first hematopoietic stem cells are generated through the hemogenic endothelium stage and are anatomically derived from the hemogenic endothelium in the dorsal aorta floor of the AGM region.

Key words : hematopoietic stem cell (HSC), hemangioblast, hemogenic endothelium, dorsal aorta

Since we, vertebrates, have acquired a closed blood-vascular system, the generation of blood cells and construction of the vascular system are closely linked both anatomically and physiologically. The first red cell activity begins in yolk sac blood islands extraembryonically. At embryonic day 7.5 (E7.5) of the mouse, blood islands are formed in the yolk sac, where inner cells are morphologically round and become primitive red blood cells. On the other hand, the surrounding cells become flat and differentiate into endothelial cells. At these sites, the appearance of primitive red blood and endothelial cells is almost simultaneous. The cells in the blood islands are considered to be bipotent cells which have the potential to differentiate into both red blood and endothelial cells. However, blood cells formed in blood islands in the yolk sac are only red blood cells (primitive erythrocytes). The first definitive hematopoietic stem cell (HSC) which has pluripotent activity to produce all types of peripheral blood cell is considered to appear intraembrionically in the AGM (aorta-gonad-mesonephros) region at about E10.5 of the mouse. The most important question here is "What is the cellular origin of this first hematopoietic stem cell?"

In vitro studies of cultured embryonic stem (ES) cells initiallly shed light on this question. They strongly suggest the presence of common precursors of hematopoietic stem and endothelial cells called hemangioblasts. During the culture of mouse embryonic stem cells, a clonal precursor, the blast colony-forming cell (BL-CFC), gives rise to blast colonies with both hematopoietic and endothelial components. Another source of support for the hemangioblast concept is that hematopoietic stem and embryonic endothelial cells express almost the same set of marker proteins and have been impossible to separate phenotypically. Anatomically also, the AGM region is composed of the dorsal aorta, germ cells, and mesonephron cells, and the dorsal aorta is an essential component for hematopoietic stem cell activity.

The hemangioblast can be isolated from differentiated ES cells based on Flk-1 expression and generates a blast colony containing hematopoietic and endothelial cells after 4 days of culture. *In vivo* studies also identified this hemangioblast in the primitive streak of the mouse embryo (Huber et al., 2004) and in the zebrafish gastrula (Vogeli et al., 2006). Surprisingly, detailed mapping studies reveal that hemangioblasts are found at the highest frequency in the posterior region of the primitive streak even before blood island development in the yolk sac. Thus, *in vitro* and *in*

vivo studies confirmed the presence of hemangioblasts in the developing embryo. A conflicting theory associates the first hematopoietic cell with a phenotypically differentiated endothelial cell having hematopoietic potential, i. e., a hemogenic endothelium. So, the next question is "How do hematopoietic cells differentiate from the hemangioblast?" A further study was conducted to clarify the precise mechanism of the generation of hematopoietic cells from hemangioblasts (Lancrin et al., 2009). According to this study, hemangioblasts generate hematopoietic cells through the formation of a hemogenic endothelium intermediate. In that study, the development of individual blast colonies was followed by time-lapse photography in order to investigate the developmental steps leading to the generation of hematopoietic cells. After 36-48 hours of culture, the hemangioblasts gave rise to a tight adherent structure, and then non-adherent round cells appeared and proliferated to generate a mature blast colony. FACS analysis shows that $Tie2^{hi}$ c-Kit^{+} $CD41^{-}$ cells can generate hematopoietic progenitors. Tie2 is used here as an endothelial marker and CD41 as cells with hematopoietic commitment. So, the endothelial nature of $Tie2^{hi}$c-$Kit^{+}$$CD41^{-}$ cells is clear. This fraction displayed a low but significant hematopoietic potential. Therefore, this fraction of cells can be called "hemogenic endothelium". At the molecular level, the transcription fractor Tal1/Scl is indispensable for the establishment of this hemogenic endothelium population. In summary, the hemangioblasts appear temporarily as hemogenic endothelium to generate definitive hematopoietic stem cells. The single cell trace method also confirmed the hemogenic endothelium origin of blood cells (Eiken et al., 2009).

So, the last question is "Where is this hemogenic endothelium formed in the developing embryo *in vivo*?" The answer comes from the observation of zebrafish development combined with fluorescent reporter transgenes and confocal timelapse microscopy (Bertrand et al., 2010). In that study, hematopoietic stem cells were derived directly from endothelial cells in the ventral wall of the dorsal aorta. Importantly, this process does not involve asymmetric cell division but a new type of cell behavior called endothelial hematopoietic transition (Kissa et al., 2010). The core binding factor Runx1 is necessary for the transition of the hemogenic endothelial cells into hematopoietic stem cells (Chen et al., 2009). The formation of these hemogenic endothelial cells seems to be only temporary.

The decades of controversy about the site and origin of the first definitive blood cells led to a conclusion, because the cellular mechanisms of hematopoietic stem cell (HSC) generation have been highly conserved across vertebrate evolution. Actually, in the mouse too, a new dissection procedure to visualize the deeply located aorta enabled us to observe the dynamic de novo emergence of hematopoietic stem cells directly from ventral aortic hemogenic endothelial cells (Boisset et al., 2010). At first, we believed that the yolk sac might be the birthplace of both primitive erythroid and hematopoietic stem cells. Since an intraembryonic definitive hematopoietic origin (AGM region) was identified, whether blood cells arise from mesodermal cells, mesenchymal progenitors, bipotent endothelial-hematopoietic precursors, or hemogenic endothelial cells remains controversial (Zovein et al., 2008). In conclusion, the hemangioblastic activity appears much earlier than previously expected in the posterior mesoderm. These hemangioblasts differentiated into a transiently hemogenic endothelium intermediate by acquiring an endothelial phenotype and migrating to the ventral floor of the dorsal aorta to produce the first hematopoietic stem cells (Fig. 1). Although these newly born hematopoietic stem cells (HSCs) enter the circulation differently (in zebrafish, AGM HSCs enter the circulation via the

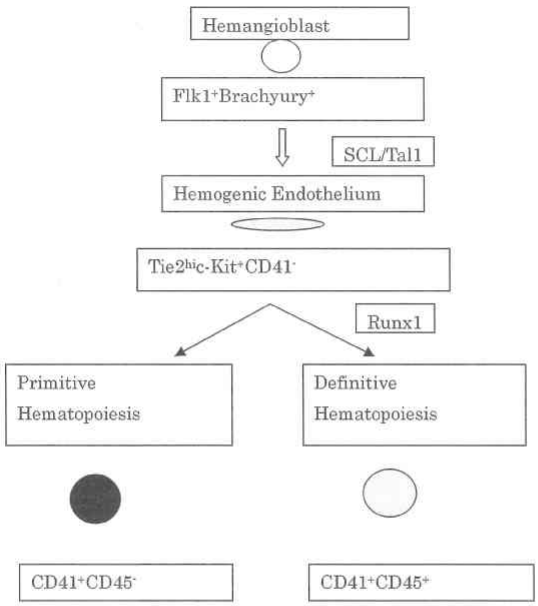
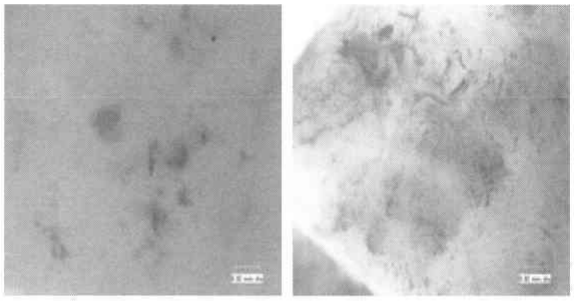


Figure 1 Stepwise differentiation of hemangioblasts into hematopoietic cells. Both primitive and definitive hematopoietic cells are produced through a hemogenic endothelium intermediate. Scl/Tal 1 is necessary for the formation of the hemogenic endothelium intermediate and Runx 1 for the beginning of definitive hematopoiesis



Lmo2-/- ES cells　　Lmo2+/- ES cells

Figure 2　(left) Murine Lmo2−/−ES cells implanted subcutaneously into a nude mouse. Note the defect of sprouting angiogenesis from hemangioblast colonies. (right) Murine Lmo2+/−ES cells in the same procedure. In both photographs, endothelial cells are labeled by Lmo 2 promoter-induced lacZ

dorsal wall of the caudal vein and, in the mouse, HSCs most likely enter the circulation directly from the arterial wall), before the first blood cell is produced, the pathway of the first blood cell has already been prepared by the endothelial cell network for a closed blood-vascular system to function.

Finally, the possible role of hematopoietic stem cells in the construction of blood vessels (angiogenesis) should be discussed. For example, the LIM domain transcription factor Lmo2 is necessary for both primitive and definitive hematopoiesis (Yamada et al., 1998). Lmo2 is highly expressed in all blood vessel endothelial cells and plays an important role in angiogenesis (Fig. 2) (Yamada et al., 2000). Interestingly, its expression is on arterial endothelium. This observation is compatible with the concept of arterial hematopoiesis. Thus, while arterial endothelial cells produce hematopoietic stem cells, hematopoietic stem cells promote angiogenesis by producing angiopoietin-1 (Takakura et al., 2000). Therefore, without blood stem cells, the vascular system cannot be completely constructed. Endothelial and blood cells depend on each other. Here again, hematopoiesis and angiogenesis are closely linked and cannot be separated.

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