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The regulatory signal for normal and abnormal hematopoiesis

The significance and application of vascular niche in the development and maintenance of hematopoietic stem cells

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Abstract
Deriving hematopoietic stem cells (HSCs) from human pluripotent stem cells is one of major goals in stem cell and hematological research. To induce HSCs from human pluripotent stem cells, many attempts to mimic embryonic development through stepwise exposure to morphogens. HSCs arise from dorsal aorta of embryos then migrate and settle in the bone marrow. Development and maintenance of HSCs are controlled by the microenvironmental cues around the blood vessels (called vascular niche) through morphogens and cytokines. Vascular niche serves as a common mechanism from embryo development to life-long maintenance of HSCs. In this chapter, I discuss that how vascular niche regulates development and maintenance of HSCs and exemplify the role of vascular niche to exquisitely induce HSCs from human pluripotent stem cells.

Keywords Hematopoietic stem cell · Pluripotent stem cell · Niche · Transcriptional regulation · Signaling

Introduction

The development and maintenance of HSCs is controlled by a microenvironment called vascular niche via signal transduction [1]. HSCs develop from a specific subset of hemogenic endothelial cells in aortic wall of embryos. Vascular endothelial cells and surrounding mesenchymal and neural cells form vascular niche facilitate conversion of hemogenic endothelial cells to HSCs through morphogens and cytokines. HSCs develop in dorsal aorta then migrate and expand in fetal liver, and finally settle down in bone marrow. Through the entire processes, the vascular niche regulates HSCs via morphogens and cytokines. The evidence of vascular niche was initially demonstrated by Morrison et al. [2], further supported by Nagasawa and Frenette [3, 4]. Independently, Li et al. proposed an osteoblast niche, but the role of osteoblastic cells in regulation of HSCs was called under question (see Sugimura review [5] for details). In this chapter, I introduce the role of vascular niche in the development and maintenance of HSCs and exemplify the application of vascular niche to induce HSCs from human pluripotent stem cells.

The role of vascular niche in the development of hematopoietic stem cells

Decades of research attempted to induce HSCs from human pluripotent stem cells by mimicking embryonic development through stepwise exposure to morphogens. HSCs arise from the ventral wall of the dorsal aorta of the embryo [6]. The specialized vascular endothelial cells (hemogenic endothelial cells) convert to HSCs, then migrate to and settle down in bone marrow via circulation. Detailed analysis of hemogenic endothelial cells in murine embryos reveals factors derived from vascular niches (Fig. 1). First, hedgehog signals specify hemogenic endothelial cells, followed by Notch signal that converts them to HSCs. WNT signal is partly involved in the conversion of hemogenic endothelial cells to HSCs. Using zebrafish as a model, TGFβ promotes conversion of hemogenic endothelial cells to HSCs, but the opposite effect is reported in both mouse and human cells. Although zebrafish model serves as a leading force to the understanding of HSC development because of its advantages in fast cycle of generation and imaging technology, it is important to note that the findings should be confirmed

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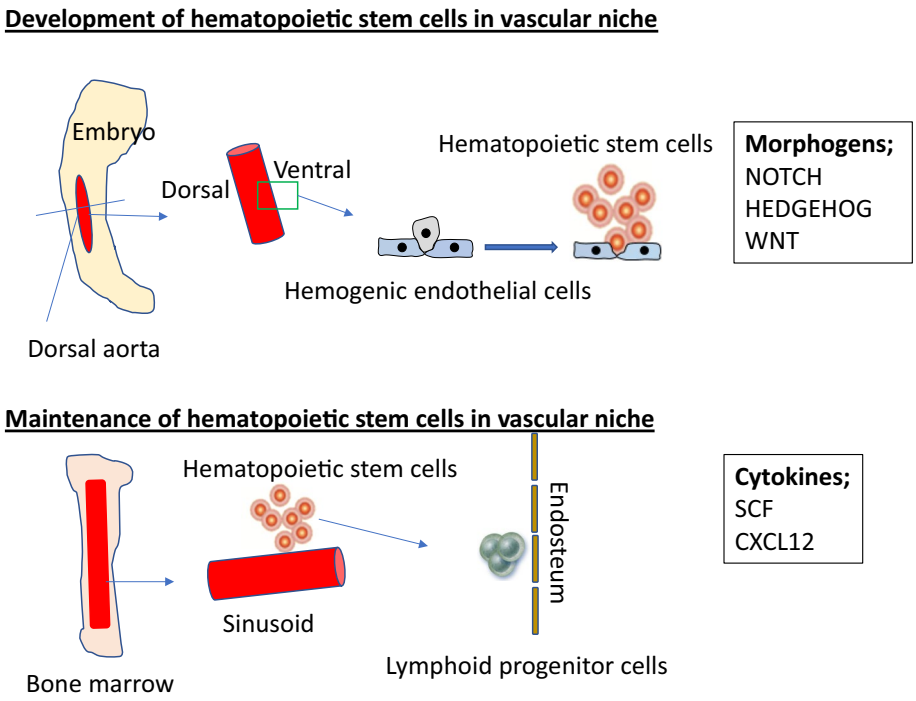

Fig. 1 Development and maintenance of hematopoietic stem cells in vascular niche. HSCs arise from hemogenic endothelial cells present on the ventral side of the dorsal aorta of the embryo. Morphogens such as Notch and Hedgehog regulate the formation of hemogenic endothelial cells and conversion to HSCs. Hemogenic endothelial cells requires transcription factors (RUNX1, HOXA, SPI1, ERG, etc.) which are master regulators of HSC development. In adult bone marrow, cytokines such as SCF and CXCL12 produced by vascular niche maintain HSCs

in mouse or human model. The identity and regulation of hemogenic endothelial cells are well described in mouse embryos, but not as much precisely studied in human. A report proved human hemogenic endothelial cells located in dorsal aorta that can be transplanted to immunodeficient murine models [7]. Signals from vascular niche involved in the development of human HSCs are still not clear. To induce HSCs from human pluripotent stem cells, the important step is to clarify the signals involved in specification and conversion of hemogenic endothelial cells to HSCs in human embryos. In recent years, RNA sequencing of hemogenic endothelial cells from human embryos was conducted by Elefanty et al., showed similarity between those from human embryos and derived from human pluripotent stem cells. [8]. The study used whole population of aorta region so did not dissociate subpopulation of human hemogenic endothelial cells that convert to HSCs. To examine the precise identity of human hemogenic endothelial cells, the further elucidation with single-cell RNA-seq would be crucial.

The role of vascular niche in maintenance of hematopoietic stem cells

Once HSCs were specified from embryos or derived from human pluripotent stem cells, they should to be maintained in vascular niche through proper combination of signaling factors. Vascular niche in bone marrow provide cytokine milieu to HSCs (Fig. 1). There are two approaches to understand how vascular niche maintain HSCs. A genetic approach using a niche cell specific Cre mouse model revealed that the cytokines SCF and CXCL12 are produced from both vascular niche endothelial cells and mesenchymal stromal cells, and these are responsible for the maintenance of HSCs. The role of various cytokines is elucidated using the same approach. For example, IL7 was produced from vascular niche endothelial cells and mesenchymal stromal cells, and involved in lymphocyte differentiation, but not in maintenance of HSCs [9]. Indeed, IL7 has been used as one of major factors to differentiate lymphocytes from HSCs. In contrast to mouse HSCs for which genetic model is available, elucidating factors that maintain human HSCs has been still challenging. Identifying such factors will be a crucial milestone to derive HSCs from human pluripotent stem cells. Recent researches showed that compounds called SR1 and UM171 maintain and expand human HSCs in culture [10]. SR1 is an antagonist of allyl hydrocarbon receptors, and its precise downstream mechanism is remained clear. The target of UM 171 is also not clear. By elucidating exquisite molecular logic of these compounds, it will be possible to employ them to maintain HSCs derived from human pluripotent stem cells. Another expected approach to understand the role of vascular niche in HSC maintenance is reconstruction of human bone marrow microenvironment [11]. This approach undertakes reconstitution of vascular niche by implanting human endothelial and mesenchymal progenitors to form ectopic human vascular niche in mouse, follow by transplantation of human HSCs. Identification of factors that maintain human HSCs will revolutionize current attempts to derive HSCs from human pluripotent stem cells. To overcome the current shortness of understanding

Table 1 Factors from vascular niche applied in inducing hematopoietic stem cells

HSC	Location	Factors	Induction from human pluripotent stem cells (Daley)	Induction from adult murine endothelial cells (Rafii)
Development	Ventral wall of dorsal aorta	Morphogens (NOTCH, HEDGHOG, WNT), Transcription factors (RUNX1, SPI1 etc.)	Morphogens + transcription factors (RUNX1, SPI1 + 5 TFs)	Transcription factors (RUNX1, SPI1 + 2 TFs)
Maintenance	Sinusoid of BM	Cytokines (SCF, CXCL12 etc.)	Autochthonous specification in BM	Co-culture with vascular niche cells

in morphogens and cytokines from vascular niche, I took a complimentary approach to induce transcription factors (RUNX1, HOXA, SPI1, ERG, etc.), master regulators of HSC development, to hemogenic endothelial cells to convert to HSCs.

Application of vascular niche—induction of hematopoietic stem cells from human pluripotent stem cells

Derivation of HSCs from pluripotent stem cells has been long-term goal of both stem cell biology and hematology field, though has been still elusive. For decades, the two major approaches have been taken independently. The first attempts to mimic embryo development through stepwise exposure to morphogens. The approach had achieved derivation of hemogenic endothelial cells as a milestone, but still did not engraft upon transplantation. The second attempts to introduce transcription factors into heterologous types of cells such as fibroblasts and differentiated blood cells. Neither method alone could induce HSCs. Therefore, I took an approach to combine these (Table 1) [12]. First, hemogenic endothelial cells were induced from human pluripotent stem cells by stepwise exposure to morphogens. Then, by comparing the expression pattern of hemogenic endothelial cells with HSCs, I identified candidate hematopoietic transcription factors that were missing in hemogenic endothelial cells. These factors contain HOXA family and RUNX1, that are known to regulate HSC development. I then introduced expression libraries of these candidate genes into hemogenic endothelial cells. Subsequently, I transplanted library-introduced cells into mouse bone marrow to reconstitute hematopoietic system. As a result, I successfully induced long-term HSCs with multi-lineage differentiation potential. Thereafter, I identified seven transcription factors (ERG, RUNX1, LCOR, HOXA5, HOXA9, HOXA10, SPI1) to be necessary for the induction of HSCs. I speculate that these transcription factors push hemogenic endothelial cells toward HSC fate, and vascular niche in bone marrow accomplished autochthonous specification of HSCs. In addition, a report from Rafii et al. succeeded in inducing HSCs from murine adult vascular endothelial cells [13]. In their method, HSCs were induced by combination of four transcription factors (FOSB, GFI 1, RUNX 1, SPI 1) and co-culture with vascular niche cells. Both approaches from Daley and Rafii et al. mimicked the embryo development of HSCs through transcription factors and vascular niche, from different starting cells (hemogenic endothelial cells derived from human pluripotent stem cells or murine adult vascular endothelial cells). It is noteworthy that RUNX1 and SPI1 are used commonly in both groups, and vascular niche provides signals to specify HSCs (either autochthonous specification in bone marrow or co-culture with vascular niche cells). Both reports exemplify how vascular niche plays a significant role in derivation of HSCs from human pluripotent stem cells, and future goal would be able to understand precise signaling. Therefore, the identification of morphogens and cytokines involved in the generation and maintenance of HSCs is still an important subject.

Conclusion

In this chapter I reviewed the vascular niche that is responsible for the development and maintenance of HSCs and exemplified how vascular niche plays a crucial role in derivation of HSCs from human pluripotent stem cells. An ultimate goal is to exquisitely reconstruct morphogens and cytokines milieu from vascular niche to specify and maintain HSCs. Future research aims to identify morphogens and cytokines produced by vascular niche and to induce HSCs entirely in vitro.

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