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Review

The evolving views of hematopoiesis: from embryo to adulthood and from in vivo to in vitro

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ABSTRACT

The hematopoietic system composed of hematopoietic stem and progenitor cells (HSPCs) and their differentiated lineages serves as an ideal model to uncover generic principles of cell fate transitions. From gastrulation onwards, there successively emerge primitive hematopoiesis (that produces specialized hematopoietic cells), pro-definitive hematopoiesis (that produces lineage-restricted progenitor cells), and definitive hematopoiesis (that produces multipotent HSPCs). These nascent lineages develop in several transient hematopoietic sites and finally colonize into lifelong hematopoietic sites. The development and maintenance of hematopoietic lineages are orchestrated by cell-intrinsic gene regulatory networks and cell-extrinsic microenvironmental cues. Owing to the progressive methodology (e.g., high-throughput lineage tracing and single-cell functional and omics analyses), our understanding of the developmental origin of hematopoietic lineages and functional properties of certain hematopoietic organs has been updated; meanwhile, new paradigms to characterize rare cell types, cell heterogeneity and its causes, and comprehensive regulatory landscapes have been provided. Here, we review the evolving views of HSPC biology during developmental and postnatal hematopoiesis. Moreover, we discuss recent advances in the in vitro induction and expansion of HSPCs, with a focus on the implications for clinical applications.

Introduction

Hematopoietic stem and progenitor cells (HSPCs) are a category of tissue stem cells, which exhibit multipotency and self-renewal capability. Historically, HSPCs are demonstrated to be present in the bone marrow (BM), evidenced by the recovery of hematopoietic system of lethally-irradiated recipients (mice and guinea pigs) after BM cell transplantation (Jacobson et al., 1951; Lorenz et al., 1951). To quantify the cells with hematopoietic reconstitution ability in the mouse BM, a statistical relationship between the number of reconstituting cells and the survival rate of recipients in a defined irradiated dose has been determined (McCulloch and Till, 1960). However, the quantification of reconstituting cells based on survival rate is indirect. Given that the transplanted BM cells are capable of proliferating and generating colonies (that appear as nodules) in the spleens of recipients, a direct measurement of the number of reconstituting cells and the according number of colonies is established (approximately a single colony derived from 10,000 transplanted BM cells) (Till and McCulloch, 1961). In addition to quantitative analysis of HSPCs in the BM, the assay for colony-forming units in spleen (CFU-S), together with karyotype analysis of irradiation-induced chromosome aberrations, demonstrates that single HSPCs can generate multi-lineage progenies (including erythrocytes, granulocytes, and megakaryocytes) (Becker et al., 1963; Wu et al., 1967). To further resolve the relationships between HSPCs and their progenies, irradiation-induced chromosome aberrations or virally integrated exogenous genes are applied as clonal markers for lineage tracing and one of the main conclusions is that differentiated hematopoietic lineages are shown to arise from multipotent or lineage-restricted HSPCs (Abramson et al., 1977; Williams et al., 1984; Dick et al., 1985; Keller et al., 1985; Lemischka et al., 1986). The aforementioned studies demonstrate the existence of HSPCs and propose a hierarchical model illustrating the HSPC differentiation pathway (Fig. 1A). However, purifying hematopoietic stem cells (HSCs) and hematopoietic

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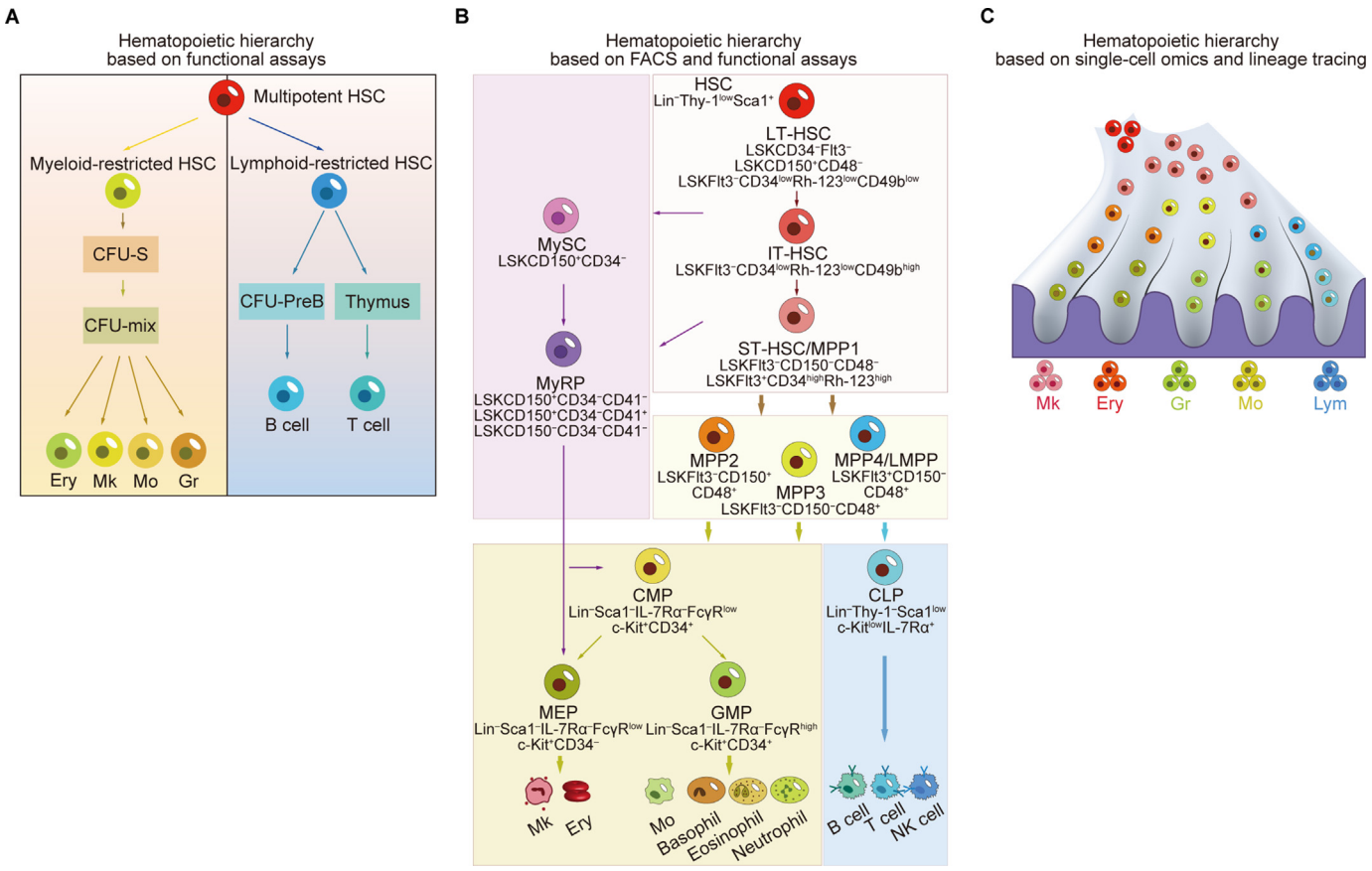

Fig. 1. The evolving hematopoietic hierarchy to describe HSC differentiation property under native and perturbed conditions. **A**: Hematopoietic hierarchy established by functional assays. **B**: Hematopoietic hierarchy established by FACS and functional assays at cell-population or single-cell levels. **C**: Hematopoietic hierarchy established by single-cell omics and lineage tracing. HSC, hematopoietic stem cell; CFU-S, assay for colony-forming units in spleen; Ery, erythrocytes; Mk, megakaryocyte; Mo, monocyte; Gr, granulocyte; pre-B, precursor of B cell; FACS, fluorescence-activated cell sorting; LT-HSC, long-term HSC; IT-HSC, intermediate-term HSC; ST-HSC, short-term HSC; MPP, multipotent progenitor; LMPP, lymphoid-primed multipotent progenitor; MySC, myeloid-restricted stem cell; MyRP, myeloid-restricted repopulating progenitor; CMP, common myeloid progenitor; MEP, megakaryocyte/erythrocyte progenitor; GMP, granulocyte/macrophage progenitor; CLP, common lymphoid progenitor; NK cell, natural killer cell; Lym, lymphocyte; LSK, Lineage$^-$Sca1$^+$c-Kit$^+$; Rh-123, rhodamine 123.

progenitor cells (HPCs, including multipotent progenitor cells [MPPs] and lineage-restricted progenitor cells) for analyses of lineage relationships and functions is unresolved.

Owing to the diverse but unique immunophenotypes of hematopoietic lineages, Weissman and colleagues pioneer the applications of monoclonal antibodies to recognize the cell surface "differentiation" (abbreviated as CD) antigens, which enables to purify HSCs from adult mouse BM at a ratio of ~0.05% or from human fetal BM at a ratio of 0.05%–0.1% (mouse: CD4$^-$CD8$^-$B220$^-$Mac-1$^-$Gr-1$^-$ [hereafter called Lin$^-$] Thy-1^{low}Sca1$^+$; human: Lin$^-$Thy-1$^+$CD34$^+$) (Muller-Sieburg et al., 1986; Spangrude et al., 1988; Baum et al., 1992). With the improvement of fluorescence-activated cell sorting, functional characterization of HSCs in vivo can reach to single-cell resolution (Smith et al., 1991). Notably, whether the individual Lin$^-$Thy-1^{low}Sca1$^+$ HSCs are functionally homogeneous remains unknown. Mitochondrial membrane-localized fluorescent vital dye rhodamine 123 (Rh-123) has been reported to distinguish Lin$^-$Thy-1^{low}Sca1$^+$ HSCs into Rh-123^{low} and Rh-123^{high} subsets, which separately represent resting and activated HSCs with different proliferative potential and clonogenic capacities (Spangrude and Johnson, 1990). Moreover, Lin$^-$Thy-1^{low}Sca1$^+$ HSCs can be divided into Lin$^-$Mac-1$^-$CD4$^-$, Lin$^-$Mac-1^{low}CD4$^-$, and Mac-1^{low}CD4^{low} subpopulations, which are featured by their declined self-renewal capability (Morrison and Weissman, 1994; Morrison et al., 1997). In addition to multipotent HSPC purification, a new type of lineage-restricted HPCs, termed as common lymphoid progenitors (Lin$^-$Thy-1$^-$Sca1^{low}c-Kit^{low}IL-7Rα$^+$), was identified to only generate T cells, B cells, and natural killer cells in the mouse BM (Kondo et al., 1997). Later on, the common myeloid progenitors (CMPs, Lin$^-$Sca1$^-$IL-7Rα$^-$FcγR^{low}c-Kit$^+$CD34$^+$) that can produce all myeloid lineages (including megakaryocyte/erythrocyte lineage-restricted progenitors [MEPs, Lin$^-$Sca1$^-$IL-7Rα$^-$FcγR^{low}c-Kit$^+$CD34$^-$] or granulocyte/macrophage lineage-restricted progenitors [GMPs, Lin$^-$Sca1$^-$IL-7Rα$^-$FcγR^{high}c-Kit$^+$CD34$^+$]) were identified in the mouse BM (Akashi et al., 2000). The multipotent HSPCs, lineage-restricted HPCs, and mature lineages collectively constitute the hematopoietic hierarchy, which serves as a blueprint for HSC biology (Zhang et al., 2018) (Fig. 1B).

The conventional technologies and methods that are applied to study HSPC biology include transplantation and assays for colony-forming units in culture (CFU-C) or CFU-S. For transplantation, donor-derived HSPCs, mixed with BM cells (named after helper cells), are delivered into recipients, which suffer from preconditioning (i.e., irradiation) for immunosuppression and HSPC pool clearance. The primary transplantation is performed to evaluate multilineage reconstitution abilities of HSPCs and the serial transplantation is employed to characterize self-renewal ability of HSCs. To identify the HSPCs with long-term reconstitution and self-renewal capabilities at clonal level, single-cell transplantation is developed. For example, single Lin$^-$Sca1$^+$c-Kit$^+$CD34$^{-/low}$ cells from adult mouse BM have been demonstrated to display myeloid–lymphoid

reconstitution and self-renewal capabilities (Osawa et al., 1996); a population of self-renewing HPCs that are directly derived from HSCs and exhibit myeloid-restricted reconstitution ability has been characterized in the adult mouse BM via single-cell transplantation (Yamamoto et al., 2013). For CFU-C, cells are cultured in a methylcellulose-based medium supplemented with defined cytokines for several days, followed by quantification of erythrocyte, megakaryocyte, granulocyte, and macrophage colonies. This method is used to assess proliferation and multilineage differentiation potency of HSPCs. Notably, replating CFU enables to evaluate self-renewal ability of HSCs in vitro. For CFU-S, cells are injected into irradiated mouse recipients, followed by counting the number of spleen nodule-like colonies derived from HSPCs. This assay is often used to characterize multilineage differentiation capability of HSPCs in vivo. Given that the transplantation or CFU-C/CFU-S alone is unable to record differentiation history of HSPCs, the combination of lineage tracing and transplantation/CFU-C/CFU-S is devised. At early stage, irradiation-induced chromosomal aberrations or virally integrated exogeneous genes act as genetic markers to show that HSPCs are the founder cells of hematopoietic system (Abramson et al., 1977; Keller et al., 1985). Later on, more efficient, convenient, and high-throughput lineage tracing tools have been developed. For example, DNA barcoding introduced by viral infection, clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9-based genome editing, transposon integration, and genetic recombination serves as stably inheritable markers to reconstruct the hematopoietic hierarchy (Lu et al., 2011; Naik et al., 2013; Sun et al., 2014; Pei et al., 2017; Alemany et al., 2018). Multicolor systems introduced by genetic recombination provide another means to track hematopoietic lineage differentiation (Ganuza et al., 2017; Henninger et al., 2017). However, the acquisition of hematopoietic lineage information is insufficient to understand the regulatory mechanisms of cell identity. To this end, multimodal single-cell omics have been developed (Baysoy et al., 2023). The computational algorithms that can decode the abundant omics data facilitate our understanding of cell heterogeneity, continuous differentiation trajectory (Fig. 1C), cell−cell interactive network, spatially resolved molecular atlas, and regulatory landscape (Stuart and Satija, 2019; Zhang and Liu, 2019). In addition to characterization of functional properties and lineage fates/states, the dynamic behaviors of hematopoietic lineages within their native microenvironment have been visualized via optimized live imaging technologies (Christodoulou et al., 2020; Upadhaya et al., 2020). Overall, the aforementioned advances in technologies and methods provide us a multi-dimensional view of the complex hematopoietic system.

In this review, we first introduce the HSC-independent and -dependent developmental hematopoiesis, with a focus on the progress in the developmental origins of hematopoietic lineages and potential causes of HSPC heterogeneity; meanwhile, we discuss the debates on the EMP origin of blood vasculatures and the main functional roles of fetal liver in HSPC development. Next, we introduce the maintenance of postnatal HSPCs and summarize the applications of multimodal omics in profiling molecular dynamics of HSPCs and their differentiation landscapes. We also discuss niche regulation of HSPC maintenance at the adult stage. Finally, we focus on the potential solutions to support in vitro induction and expansion of HSPCs.

Developmental hematopoiesis

HSC-independent hematopoiesis

During vertebrate embryogenesis, developmental hematopoiesis is an evolutionarily-conserved process and begins with HSC-independent hematopoietic waves. A seminal study on mouse embryos showed that the earliest hematopoietic activity is detected in an extra-embryonic site, namely yolk sac, during gastrulation (Moore and Metcalf, 1970). This wave of hematopoiesis is termed primitive hematopoiesis. Within the yolk sac at embryonic day (E) 7.0−7.5, endothelial cells (ECs) and hematopoietic lineages (including primitive erythrocytes, primitive myelocytes, and megakaryocytes) that are both derived from mesoderm form a specialized structure, namely blood island (Ferkowicz and Yoder, 2005) (Fig. 2A and 2B). Beginning at the E8.25, a subset of ECs that are located in the yolk sac vasculature acquires hemogenic potential and then generates lineage-restricted HPCs through a trans-differentiation process, namely endothelial-to-hematopoietic transition (EHT). This wave of hematopoiesis is called pro-definitive hematopoiesis. The representative lineage-restricted HPCs generated in this wave include erythro-myeloid progenitors (EMPs) (Palis et al., 1999, 2001), immune-restricted/lymphoid-primed progenitors (Boiers et al., 2013), and neonatal repopulating HSCs (Yoder et al., 1997) (Fig. 2C). Later on, the hematopoietic activity initiates in an intra-embryonic site, namely para-aortic splanchnopleure (P-Sp), at around E9.0 (Cumano et al., 1996, 2001) and peaks in the dorsal aorta of aorta−gonad−mesonephros (AGM) region from E10.0 onwards (Medvinsky and Dzierzak, 1996; Sanchez et al., 1996) (Fig. 2D). Distinct from the yolk sac, the intra-embryonic sites can produce MPPs via EHT (Dignum et al., 2021; Patel et al., 2022; Yokomizo et al., 2022).

Evolving view on the earliest specification of hematopoietic lineages

How mesodermal cells are specified into hematopoietic lineages is a long-standing issue. Previous studies in zebrafish and mouse embryos have identified a mesodermal derivative, namely hemangioblast, serving as a common precursor for endothelial and hematopoietic lineages (Huber et al., 2004; Vogeli et al., 2006). In zebrafish embryos, the early evidence supporting the presence of so-called hemangioblasts is revealed via fluorescent labeling-mediated cell tracing (Vogeli et al., 2006). Then, the hemangioblasts are specifically defined by the expression of *npas4l* (encoding a PER−ARNT−SIM-domain-containing basic helix−loop−helix transcription factor). The *npas4l*$^{+}$ hemangioblasts sit upstream of *etv2*$^{+}$/*tal1*$^{+}$ endothelial and hematopoietic progenitors, which further generate endothelial and hematopoietic lineages (Reischauer et al., 2016). Mechanistically, Fli1, a member of ETS transcription factor family, has been shown to be a core of transcriptional hierarchy governing hemangioblast development in the embryos of zebrafish and *Xenopus* (Liu et al., 2008). In addition to serving as a hemangioblast marker, Npas4l has been shown to be a master regulator which is able to orchestrate a cohort of endothelial/hematopoietic transcription factors, such as Etv2, Tal1, and Lmo2, via direct binding (Marass et al., 2019). In mouse embryos, the hemangioblasts have been shown to be a mesodermal subset that is characterized by the co-expression of *Brachyury* (also known as *T*, encoding a T-Box transcription factor) and *Flk-1* (also known as *Kdr*, encoding a vascular endothelial growth factor receptor). This subset can be detected in the posterior primitive streak at E7.5 and further migrates into the yolk sac in which it is specified into endothelial and hematopoietic lineage (Huber et al., 2004). In 2009, Lancrin et al. (2009) demonstrated that hemangioblasts generate hematopoietic lineages via an intermediate stage of hemogenic endothelial cells (HECs). The presence of HECs has been supported by the evidence found in embryonic stem cell (ESC) culture and gastrulating embryos in mice (Lancrin et al., 2009). To reconstruct the developmental trajectory from mesoderm to primitive hematopoietic lineages, Pijuan-Sala et al. (2019) have performed single-cell transcriptome analysis of mouse gastrulation. A putative roadmap successively covering mesodermal cell, hemato-endothelial progenitor (that potentially

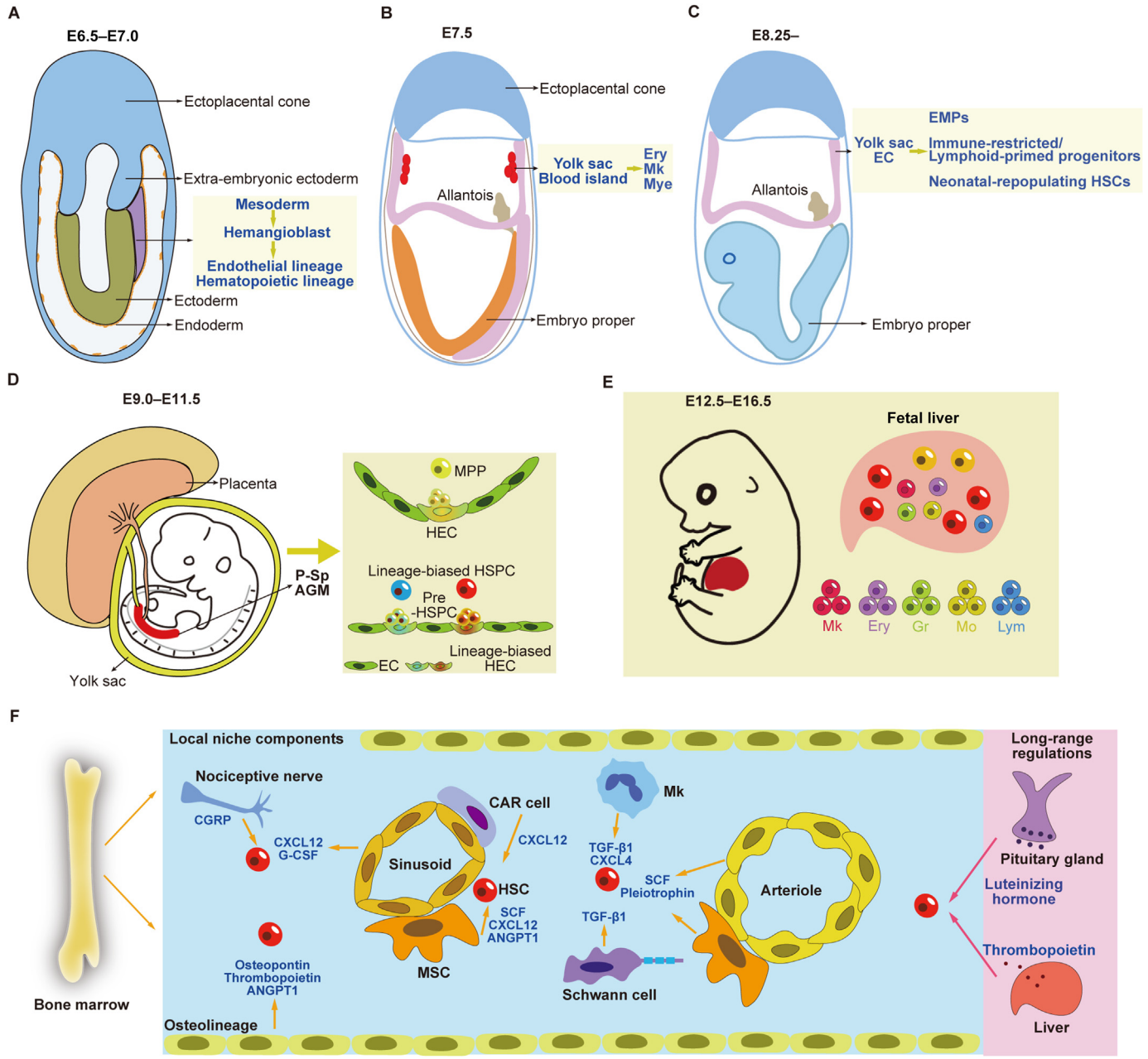

Fig. 2. Developmental and postnatal hematopoiesis across different sites in mice. **A** and **B**: The lineage specification of mesoderm-derived hemangioblasts and the generation of primitive hematopoietic lineages in the yolk sac at E6.5–E7.5. **C**: The generation of lineage-restricted HPCs in the yolk sac from E8.25 onwards. **D**: The generation of multipotent or lineage-biased HSPCs in the dorsal aorta of AGM region at E9.0–E11.5. **E**: The maturation, expansion, and differentiation of HSPCs in the fetal liver at E12.5–E16.5. **F**: The maintenance of postnatal HSPCs in the bone marrow via interactions with local niche components and distant organs. EC, endothelial cell; EMP, erythro-myeloid progenitor; P-Sp, para-aortic splanchnopleure; AGM, aorta–gonad–mesonephros; HEC, hemogenic EC; HSPC, hematopoietic stem and progenitor cell; pre-HSPC, precursor of HSPC; CGRP, calcitonin gene-related peptide; CXCL12, C–X–C motif chemokine ligand 12; G-CSF, granulocyte colony-stimulating factor; ANGPT1, angiopoietin 1; MSC, mesenchymal and stromal cell; SCF, stem cell factor; CAR cell, CXCL12-abundant reticular cell; CXCL4, C–X–C motif chemokine ligand 4; TGF-β1, transforming growth factor-β1.

includes hemangioblast), blood progenitor and EC, and primitive blood cells has been profiled. Notably, the primitive hematopoietic lineages are shown to be differentiated from hemato-endothelial progenitors and blood progenitors; meanwhile, this process does not undergo an HEC stage and thus is distinct from the EHT during definitive hematopoiesis (Pijuan-Sala et al., 2019). Recently, using single-cell transcriptomics and in vivo barcoding-based lineage tracing, founder cells that produce the primitive hematopoietic lineages, the endothelial lineages, and the mesenchymal lineages have been uncovered. The founder cells include three distinct progenitors with dual-lineage differentiation potency: the hemangioblast, the mesenchymoangioblast (that produces mesenchymal lineages and endothelial lineages), and a previously unrecognized cell type, hematomesoblast (that produces primitive hematopoietic lineages and mesenchymal lineages) (Biben et al., 2023). Mechanistically, Etv2 and Tal1 have been reported to be key transcription factors for mouse hemangioblast development (Lee et al., 2008; Lancrin et al., 2009; Pijuan-Sala et al., 2019). Recently, a T-box transcription factor-encoding gene *Eomes* has been shown to be transiently expressed in extra-embryonic mesodermal progenitors, which can

generate hematopoietic cells and ECs in the yolk sac. Eomes governs the accessibility of Tal1-bound *cis*-regulatory elements that regulate primitive erythrocyte development (Harland et al., 2021). Taken together, these findings provide updated views on the developmental origins and regulatory mechanisms of the earliest specification of hematopoietic lineages.

Debates on the contribution of EMPs to blood vasculatures

EMPs are a type of important lineage-restricted HPCs that produce erythrocytes, megakaryocytes, and myelocytes (e.g., tissue-resident macrophages) (Frame et al., 2013). In the mouse yolk sac, EMPs generated in the early wave (at about E7.5) are featured by the expression of *Csf1r* (encoding colony-stimulating factor 1 receptor) and produce brain-resident macroglia and epidermis-resident Langerhans cells (Ginhoux and Guilliams, 2016). EMPs generated in the late wave (at about E8.25) are featured by the expression of *Myb* and colonize into the fetal liver where EMPs differentiate into monocytes. The monocytes further seed multiple tissues (except for brain) to generate tissue-resident macrophages from fetal stage to adulthood (Hoeffel et al., 2015). In addition to a substantial contribution to hematopoietic lineages, EMPs have been reported to display robust fate plasticity (Iruela-Arispe, 2018). By employing genetic lineage tracing with *Cre/loxP* system mediated fluorescent labeling, mouse *Csf1r*-expressing EMPs have been found to develop into blood vascular ECs in the embryonic yolk sac, brain, lung, heart, and liver (Plein et al., 2018). This unexpected finding reveals the hematopoietic origin of embryonic vascular ECs. However, another study argues against the contribution of EMPs to vascular ECs. By employing similar or different lineage tracing strategies in mice, EMP-derived ECs have not been identified in multiple developing organs (Feng et al., 2020). To resolve this apparent controversy, here we compared the methodology and conclusions of both findings. For the genetic lineage tracing tool, Plein et al. (2018) used *Csf1r-Mer-iCre-Mer* and *Csf1r-iCre* transgenic lines, while Feng et al. (2020) used a newly-generated *Csf1r-ires-iCre* knock-in mouse line. Although the specificity of transgenic lines has been validated in each case, the knock-in line can avoid the leakage of promoter-driven *Cre/loxP* recombination and thus faithfully report the endogenous gene activity, further enhancing the specificity of lineage tracing tools. Moreover, Feng et al. (2020) found that *Kit* is also expressed in ECs in addition to EMPs, suggesting that *Kit-CreER* mediated tracing used by Plein et al. (2018) for EMP-derived ECs might not be cell type-specific. For the detection of lineage outputs, the immunostaining patterns of vasculatures, the usage of cell type-specific markers, and the methods of morphological observation are differently used between these two studies, possibly also responsible for the inconsistent conclusion. More importantly, both studies lack time-lapse imaging to show the transition from EMPs to ECs in real time, which can provide solid and direct evidence to resolve the controversial phenomenon. Overall, whether the fate transition from hematopoietic lineage to endothelial lineage can occur in vivo awaits further investigation.

HSC-dependent hematopoiesis

Shortly after or concomitant with HPC generation in the P-Sp and dorsal aorta, multipotent and self-renewing HSCs emerge in the dorsal aorta of AGM region, placenta, and other major arterial regions, such as vitelline and umbilical arteries (Medvinsky and Dzierzak, 1996; Sanchez et al., 1996; de Bruijn et al., 2000; Rhodes et al., 2008). This wave of hematopoiesis is called definitive hematopoiesis. Several studies on zebrafish and mouse embryos showed that HSC generation is through EHT (Bertrand et al., 2010; Boisset et al., 2010; Kissa and Herbomel, 2010), which has also been recapitulated in human embryos via single-cell transcriptomics (Zeng et al., 2019; Calvanese et al., 2022). In zebrafish embryos, from 24 hours post fertilization (hpf) to 60 hpf, HECs undergo EHT to generate HSPCs, which are then detached from the ventral wall of dorsal aorta and enter into the venous vessel (Zhang and Liu, 2011; Zhang et al., 2013). The generation of HSPCs involves the dynamic change of endothelial and hematopoietic programs, which is orchestrated by both signaling pathways and epigenetic modifications (He et al., 2015; Zhang et al., 2015, 2017; Liu et al., 2019; Heng et al., 2020; Ding et al., 2021). Later on, the circulating HSPCs are guided to migrate into the caudal hematopoietic tissue (CHT) by macrophages (Li et al., 2018). Within the CHT, HSPCs reside in the perivascular niche for expansion and differentiation (Xue et al., 2017, 2019; Xia et al., 2021). After transient retention in the CHT (from 2 to 4 days post fertilization), HSPCs home to the kidney marrow for lifelong hematopoiesis (Murayama et al., 2006) and lymphoid progenitors seed the thymus for lymphopoiesis (Wang et al., 2015; Lu et al., 2020). In mouse embryos, at around E9.5−E10, a subset of ECs in the dorsal aorta of AGM region is specified into HECs, followed by budding off to form multiclonal intra-aortic hematopoietic clusters (IAHCs) at E10.5−E11.5. Within the IAHCs, immature precursors of HSCs (pre-HSCs) are classified into pro-HSCs, type I pre-HSCs, and type II pre-HSCs (Taoudi et al., 2008), representing a maturation continuum (Boisset et al., 2015; Zhou et al., 2016) (Fig. 2D). Moreover, the immature pre-HSCs can be found in the extra-embryonic arterial vessels (Gordon-Keylock et al., 2013). After undergoing a significant expansion in the IAHCs, the immature pre-HSCs migrate into the fetal liver where they experience further maturation into functional definitive HSCs (Rybtsov et al., 2016). Within the fetal liver, HSCs continuously undergo expansion and lineage differentiation (Fig. 2E), followed by egression and colonization into the thymus or BM (Fig. 2F). BM-resident HSCs serve as a reservoir for lifelong hematopoiesis.

The contribution of EC/HEC heterogeneity to HSPC heterogeneity

During pro-definitive and definitive hematopoiesis, the lineage outcomes of EHT are diverse and include lineage-restricted HPCs, MPPs, and multipotent and self-renewing HSCs. Whether the lineage divergence after EHT can be attributed to the differences in ECs/HECs is becoming a hot topic. Mounting studies on avian models showed that intra-embryonic endothelial lineages arise from two distinct mesodermal derivatives, i.e., the lateral plate mesoderm and paraxial mesoderm (Pardanaud et al., 1996). The lateral plate mesoderm generates ECs forming the lining of the ventral wall of the aorta, and vasculatures of visceral organs (Pardanaud and Dieterlen-Lievre, 1999). Paraxial mesoderm-derived somite gives rise to ECs constituting the lining of the dorsal wall of the aorta, and vasculatures of the body wall, limbs, and kidney (Ambler et al., 2001; Pouget et al., 2006). Notably, several studies on zebrafish revealed a complex developmental origin of the aortic lining, which has been shown to be purely derived from lateral plate mesoderm (Jin et al., 2005; Kohli et al., 2013) or partially contributed by the somite-derived ECs (Nguyen et al., 2014). In addition, ECs in the yolk sac and placenta, two highly vascularized extra-embryonic organs in mammals, are derived from extra-embryonic mesoderm and allantoic mesoderm, respectively (Mikkola and Orkin, 2006; Enciso et al., 2010; Harland et al., 2021). In E8.5 mice, by analyzing the transcriptomes of single ECs from yolk sac, allantois, and embryo proper, the acquisition of allantois-specific transcriptional signatures was observed in allantois-derived ECs (Ibarra-Soria et al., 2018), indicating that the EC transcriptional heterogeneity is associated with the anatomical sites (Pijuan-Sala et al., 2019). Moreover, for the non-allantoic ECs, yolk sac-derived ECs are characterized by high expression levels of *Pecam1* and *Cdh5* (mature EC marker) and low levels of *Etv2* (immature EC marker), indicating that ECs generated in different

anatomical sites exhibit distinct degrees of maturity (Ibarra-Soria et al., 2018). Furthermore, Hou et al. (2022) performed single-cell transcriptomics with vascular ECs from E8.0−E11.0 mouse embryos and found that the transcriptomic feature of embryo proper-derived ECs is different from that of yolk sac-derived ECs. Specifically, the arteriovenous characteristics of the former is more apparent than that of the latter, suggesting that the reshaping of EC molecular features is jointly controlled by the anatomical sites and developmental origins. For the HECs, the differences between HEC and non-HEC manifest as chromatin states (chromatin accessibility) (Zhu et al., 2020), transcriptome states (enrichment of key transcription factors and signaling pathways) (Swiers et al., 2013; Gao et al., 2018), and immunophenotypes (Hou et al., 2020). Mounting studies have focused on resolving the heterogeneity of HECs in different anatomical sites of zebrafish and mouse embryos (Hadland et al., 2004; Yzaguirre and Speck, 2016; Tian et al., 2017; Gao et al., 2018; He et al., 2020). For example, a study showed that yolk sac-derived HEC and AGM-derived HEC require CBFβ-Runx1 regulatory elements in spatio-temporally distinct manners; meanwhile, AGM-derived HEC is specifically characterized by *Ly6a* expression and differs from yolk sac-derived HEC in the immunophenotype (Chen et al., 2011). Another lineage tracing study demonstrated that yolk sac-derived HEC is specifically labeled by the *Lyve1* (encoding lymphatic vessel endothelial hyaluronan receptor-1) marker, which shows no expression in AGM-derived HEC (Lee et al., 2016). Recently, several studies in zebrafish embryos have shown that HEC heterogeneity can contribute to lineage skews of nascent HSPCs. Spi2, a member of previously undescribed ETS transcription factor family, has been demonstrated to label myeloid/lymphoid-primed HECs that can give rise to myeloid/lymphoid-biased HSPCs (Xia et al., 2023). In addition, microRNA-223-mediated N-glycome and microRNA-128-mediated Wnt/Notch signaling direct the generation of lineage-biased HSPCs via EHT (Kasper et al., 2020; Ghersi et al., 2023). Taken together, these findings reveal a new explanation for HSPC heterogeneity and provide new insights into cell fate transitions through different layers of molecular regulations.

Revisiting the functional roles of fetal liver

Fetal liver is a mammal-specific hematopoietic organ that has long been considered to support HSPC expansion transiently (Ema and Nakauchi, 2000). In mouse embryos, from E11.5 to E12.5, the number of functional HSCs is increased from 1−2 to 50−60; from E12.5 to E16.5, the pool of HSCs is expanded for approximately 38 folds (Ema and Nakauchi, 2000; Kumaravelu et al., 2002). Mechanistically, the functional role of fetal liver in supporting HSPC expansion is dependent on the niche component-derived pro-proliferative cytokines (Gao and Liu, 2018), geometry of portal vessel niche (Khan et al., 2016), and functional hubs of HSPC expansion (e.g., pocket-like units [Tamplin et al., 2015; Gao et al., 2022]). However, the notion that fetal liver serves as a hematopoietic organ mainly supporting HSPC expansion has been continuously revised. In 2016, Rybtsov et al. (2016) demonstrated that pre-HSCs initially undergo a dramatic expansion in the AGM region and then mature into definitive HSCs in the mouse fetal liver (Rybtsov et al., 2016). The expansion of pre-HSC pool prior to fetal liver colonization accounts for the number burst observed in the fetal liver. In addition to supporting the maturation of pre-HSCs, fetal liver has been reported to support the maturation from precursors of HPCs to multiple HSC-independent HPCs (e.g., MPP, CMP, GMP, and MEP) (Yokomizo et al., 2022). Moreover, a recent study on mouse embryos using a multicolor lineage tracing tool has shown that the extent of HSPC expansion supported by the fetal liver is overestimated and that HSPC expansion is modest (about two folds) (Ganuza et al., 2022). Furthermore, fetal liver has also been shown to be an important native niche supporting HSPC differentiation (Hoeffel et al., 2015; Ganuza et al., 2022). Taken together, these findings revise our understanding of the functional role of fetal liver during mammalian fetal hematopoiesis.

Postnatal hematopoiesis

At the late gestation in mammals, HSPCs exit from the fetal liver and home to the BM. Upon BM colonization, HSPCs are responsible for the lifelong maintenance of hematopoiesis. From embryonic development to postnatal stage, HSPCs have been reported to undergo significant transitions in cell states and functional properties, such as cell cycle status and lineage differentiation potentials under native and post-transplantation conditions (Chen et al., 2019). Moreover, to sustain a stable turnover rate of blood cells after birth, HSPCs differentiate into distinct mature hematopoietic lineages. The state and functional transitions of HSPCs are orchestrated by the dynamic changes of molecular signatures (McKinney-Freeman et al., 2012; Chen et al., 2019; Roy et al., 2021). The collection of three-dimensional (3D) genomes, epigenomes, transcriptomes, and RNA epigenomes provides an opportunity to jointly analyze multi-modal molecular information. The mechanistic insights into the state and functional transitions of HSPCs at transcriptional and post-transcriptional levels serve as a paradigm for uncovering principles of stem cell biology (Orkin and Zon, 2008).

Compared to the transient hematopoietic tissues/organs at embryonic and fetal stages, BM is a long-term harbor for HSPC activity across the late gestation and postnatal stage. BM not only protects HSPCs against external hazards but also provides a signaling hub for their quiescence, self-renewal, and differentiation (Pinho and Frenette, 2019).

Molecular feature dynamics of postnatal hematopoiesis

Chromosome architecture profiling

Chromosomes are folded in the nucleus and form well-organized spatial architectures (Bonev and Cavalli, 2016). The architecture of 3D chromosomes includes chromosomal compartments, topologically associating domains, and *cis*-regulatory element interactions, which are all tightly associated with gene transcription regulations (Cremer and Cremer, 2001). To reveal the dynamics of chromosome architecture and its association with cell fate transitions, a low-input method, called tagmentation-based Hi-C, that is applied to capture chromosomal 3D organization has been devised (Zhang et al., 2020). For the analysis of HSC differentiation landscape, 10 major hematopoietic cell types, including multipotent HSPCs, lineage-restricted HPCs, megakaryocytes, and granulocytes, from adult mouse BM were collected based on their immunophenotypes. Consequently, the switching from B compartment (that is correlated with inactive gene expression) to A compartment (that is correlated with active gene expression) has been characterized during differentiation from HSCs to granulocytes, supporting a gradual transcriptional activation and high expression level of granulocyte-specific genes. Moreover, the condensed chromosome architecture and the decreased long-range chromatin interactions are shown to be unique features of terminally differentiated lineages. Furthermore, the chromosomal architecture in the highly expressed genes folds into a specialized structure, namely gene-body-associating domain, in which there exist frequent gene body interactions. Of note, the organization of gene-body-associating domain is dynamic during HSC differentiation and is highly associated with gene expression activation (Zhang et al., 2020).

Chromatin accessibility profiling

Chromatin accessibility is one of the regulatory logics of gene transcription. The accessible *cis*-regulatory elements bound by

trans-acting factors contribute to an active gene transcription; therefore, chromatin accessibility is an important feature for cell identity (Klemm et al., 2019). To characterize the chromatin states of *cis*-regulatory elements underlying cell fate determinations, an assay for transposase-accessible chromatin by sequencing at single-cell level has been developed (Buenrostro et al., 2015). For the analysis of chromatin state dynamics of HSC differentiation, nine types of hematopoietic lineages, including multipotent HSPCs, lineage-restricted HPCs, plasmacytoid dendritic cells, and monocytes from human BM were collected based on immunophenotypes (Buenrostro et al., 2018). By profiling chromatin accessibility landscape, the differentiation from HSCs to downstream lineages is shown to be a continuous process. More importantly, integration of chromatin accessibility landscape and gene expression profiles reveals a coordinately dynamic change among *cis*-regulatory element accessibility, gene expression signatures, and cell differentiation (Buenrostro et al., 2018). Recently, by profiling chromatin accessibility landscape of HSPCs from adult mouse BM, lineage priming and fate restriction of HSPCs has been shown to be predetermined by chromatin states of *cis*-regulatory elements, not by transcriptional priming (Meng et al., 2023).

Histone modification profiling

Histone modifications, including active modifications and repressive modifications, play a critical role in determining chromatin states and thereby have an impact on gene expression. To resolve the relationship between histone modifications and lineage fate determination, the single-cell chromatin immunocleavage method has been developed and leveraged to analyze HSC differentiation landscape (Zeller et al., 2023). Different BM sub-populations that contain HSPCs were sorted by flow cytometry from adult mice. For the analysis of active H3K4me1 and H3K4me3 modifications, lineage choices at the HSPC stage have been demonstrated to be made at chromatin level by acquiring active modifications. The configuration of active modifications is mediated by key transcription factors specifying certain lineages. For the analysis of repressive H3K9me3 and H3K27me3 modifications, fate restrictions from HSPCs to mature lineage cells (regardless of lineage types) after lineage choices depend on the repressive modifications (Zeller et al., 2023).

DNA methylation profiling

DNA methylation located at transcription start sites has been considered to be a repressive marker for gene transcription regulations (Jones, 2012). To understand the dynamic change of DNA methylation during cell differentiation, single-cell DNA methylome information has been collected from multipotent HSPCs, lineage-restricted HPCs, and differentiated hematopoietic lineages in humans at adult stage (Farlik et al., 2016). Consequently, the myeloid–lymphoid lineage choice of HSPCs was shown to be marked by DNA methylation on the key transcription factor-binding sites. Moreover, when joint analysis of DNA methylation, gene expression, histone modification, and chromatin accessibility was performed, these signatures are well orchestrated to maintain a proper transcription activity and a stable cell fate determination (Farlik et al., 2016). In another study on HSPC differentiation in adult mice, the differential CpG enrichment in the key transcription factor-binding sites has been demonstrated to be a determinant of functional specificity (i.e., hematopoietic lineage choice) of DNA methylation (Izzo et al., 2020).

Transcriptomic profiling of postnatal HSPC states

Single-cell transcriptomics enables to capture transcriptional signatures from individual cells at the different life stages. Recently, Cheng and colleagues have collected information of transcriptomes and chromatin states from HSPCs in humans at neonatal, infant, child, and adult stages (Zhang et al., 2022). By analyzing the transcriptional dynamics, the transcriptional activity has been shown to be enhanced from neonatal stage onwards and the transcriptional features have been demonstrated to be determined at early stage (before infant stage) after birth. Moreover, erythroid differentiation potential of HSPCs evidenced by gene expression and colony-forming ability has been reported to be enhanced after the infant stage (Zhang et al., 2022), which is likely associated with the high turnover rate of erythroid lineages in childhood and adulthood (Sender and Milo, 2021). Overall, this study provides a valuable resource for state characterization of postnatal HSPCs, which are more available to be clinically intervened compared to developing HSPCs.

RNA m^{6}A modification profiling

RNA epigenetics is an emerging regulatory logic at the post-transcriptional level. N^6-methyladenosine (m^{6}A) is an abundant modification on mRNAs and plays an important role in determining fates of mRNAs (Shi et al., 2019). To reveal the dynamic m^{6}A modification landscape during HSPC differentiation, a sequencing method to map transcriptome-wide m^{6}A-modified mRNAs has been devised (Zhang et al., 2022). The patterns of m^{6}A modification in HSC differentiation landscape have been shown to depend on inheritance from developmental stages or de novo establishment. From long-term HSCs to short-term HSCs, the m^{6}A modification patterns show a dramatic alteration, suggesting that m^{6}A plays a critical role in regulating HSC quiescence and identity maintenance (Yao et al., 2018; Cheng et al., 2019). Moreover, in multipotent HSPCs and committed lineage-restricted HPCs, m^{6}A level shows different correlations with mRNA level, suggesting distinct functional roles and regulatory mechanisms of m^{6}A in balancing stem cell multipotency and lineage commitment (Lee et al., 2019).

Niche regulations of postnatal hematopoiesis

During postnatal hematopoiesis in mammals, BM acts as a crucial microenvironment for quiescence, self-renewal, differentiation, and migration of HSPCs. The niche components of BM are divided into non-hematopoietic components and hematopoietic lineages. The former mainly includes ECs, perivascular mesenchymal and stromal cells, adipocytes, osteolineage cells, glial cells, and nerves (e.g., sympathetic nerve and nociceptive nerves); the latter primarily contains megakaryocytes, macrophages, and regulatory T cells (Pinho and Frenette, 2019). The application of single-cell transcriptomics to profile BM niche constituents enables us to deeply understand heterogeneity of the main niche cell types, such as ECs, perivascular mesenchymal and stromal cells, and osteoblasts (Tikhonova et al., 2019).

Although BM-resident HSPCs egress out of the vasculatures for maintenance of quiescence, self-renewal, and lineage commitment, the ECs still serve as an indispensable cell source of secretory factors responsible for HSPC maintenance. In the postnatal BM niche, Morrison and colleagues have shown that HSCs at the early postnatal stage (at postnatal days 4 and 14) are located proximately to sinusoidal blood vessels. Moreover, EC-derived stem cell factor (SCF) plays a critical role in HSC maintenance (Kara et al., 2023). In the adult BM, ECs and EC-derived SCF, C–X–C motif chemokine ligand 12 (Cxcl12), and pleiotrophin have been shown to be also required for HSC maintenance (Ding et al., 2012; Ding and Morrison, 2013; Himburg et al., 2018). Of note, non-dividing HSCs are shown to locate within 10 μm of sinusoidal blood vessels; most of BM-resident HSCs are away from arteriolar blood vessels (Acar et al., 2015). Given that HSCs are distributed into perivascular niche in the BM, perivascular mesenchymal and stromal cells have been extensively characterized. The perivascular mesenchymal and stromal cells have been featured by the expression of *Nestin* (Mendez-Ferrer et al., 2010), *Lepr* (encoding leptin receptor) (Ding et al., 2012; Yue et al., 2016), and *Ng2*

(encoding neural–glial antigen 2) (Kunisaki et al., 2013) or defined as Cxcl12-abundant reticular stromal cells (Sugiyama et al., 2006). Both in the early postnatal hematopoiesis and adult hematopoiesis, perivascular mesenchymal and stromal cells are required for HSC maintenance and differentiation via secreting SCF, Cxcl12, angiopoietin 1 (ANGPT1), interleukins, osteolectin, and vascular cell adhesion molecule 1 (Pinho and Frenette, 2019; Shen et al., 2021; Kara et al., 2023). Adipocytes and oeteolineage cells share a common developmental origin, namely skeletal stem cells. Skeletal stem cells bifurcate into adipogenic progenitors and osteogenic progenitors, which further generate adipocytes and oeteolineage cells, respectively (Zhou et al., 2014, 2017). Adipocytes have been reported to be harmful to adult HSC function but promote recovery and regeneration of impaired hematopoietic system (Kricun, 1985; Zhou et al., 2017). For the oeteolineage cells, BM imaging showed that HSCs are preferentially located in the central area of BM and distant from bone surfaces (Acar et al., 2015). The factors, osteopontin (Stier et al., 2005), thrombopoietin (Qian et al., 2007; Yoshihara et al., 2007), and ANGPT1 (Arai et al., 2004), that are involved in regulations of HSC function can be secreted by oeteolineage cells. The neural regulation of HSPC function has been widely reported (Agarwala and Tamplin, 2018). Recently, the nociceptive nerve has been demonstrated to be required for HSC mobilization; meanwhile, in collaboration with sympathetic nerve, the nociceptive nerve can maintain HSCs in the mouse BM (Gao et al., 2021). In addition to the local niche components, the pituitary gland has been shown to restrict excessive expansion of murine HSCs at the puberty stage through secreting luteinizing hormone (Peng et al., 2018); the liver in adult mice has been reported to produce thrombopoietin to maintain BM HSCs in a long-range regulatory manner (Decker et al., 2018). Taken together, by combining omics and functional analyses, more unknown niche components and their uncharacterized functional roles in maintaining postnatal HSPCs can be revealed (Fig. 2F).

In vitro hematopoiesis

By recapitulating the biological processes of generation, expansion, and maintenance of HSPCs in vivo, in vitro culture systems/platforms for induction of immunocompetent HSPCs from ESCs/pluripotent stem cells (PSCs) and long-term expansion of HSPCs have been developed. These advances hold a great promise for stem cell-based therapies of hematological malignances.

Induction of immunocompetent HSPCs

The strategies to induce immunocompetent HSPC generation include stepwise differentiation of ESCs/PSCs, trans-differentiation of ECs or fibroblasts, and de-differentiation of lineage-committed HPCs (Fig. 3A). For the stepwise differentiation of ESCs/PSCs, many efforts have been devoted to establishing stable culture/co-culture conditions with defined morphogens/cytokines/growth factors, which facilitate the acquisition of hematopoietic potential in ESCs/PSCs-derived embryoid bodies (Nakano et al., 1994; Kaufman et al., 2001; Chadwick et al., 2003; Kennedy et al., 2007; Ditadi et al., 2015). Moreover, the ectopic expression of genes has been performed to improve the ability to induce functional HSPCs (Kyba et al., 2002; Rideout et al., 2002; Davidson et al., 2003). However, these protocols/methods still unable to generate functional HSPCs with robust lymphoid reconstitution capability. In 2017, Sugimura et al. (2017) combined directed differentiation of human PSC-derived embryoid bodies and transcription factor overexpression to generate HSPCs. More importantly, the induced HSPCs exhibit multilineage reconstitution ability in the primary mouse recipients and self-renewal ability in the secondary mouse recipients. For the trans-differentiation-based strategy, in 2014, Rafii and colleagues generated a method to convert human non-hemogenic ECs into MPPs without a pluripotent intermediate (Sandler et al., 2014). Specifically, this method combines transcription factor expression and vascular niche induction to endow EC-derived hematopoietic cells with multilineage differentiation (without differentiation ability of T cells) and self-renewal abilities (Sandler et al., 2014). Later on, by further optimizing the induction strategy, a method to convert mouse non-hemogenic ECs into HSCs bypassing the pluripotent intermediate has been devised. The induced HSCs share similar transcriptome features and functional properties to that of primary adult HSCs (Lis et al., 2017). For the de-differentiation-based strategy, Riddell et al.

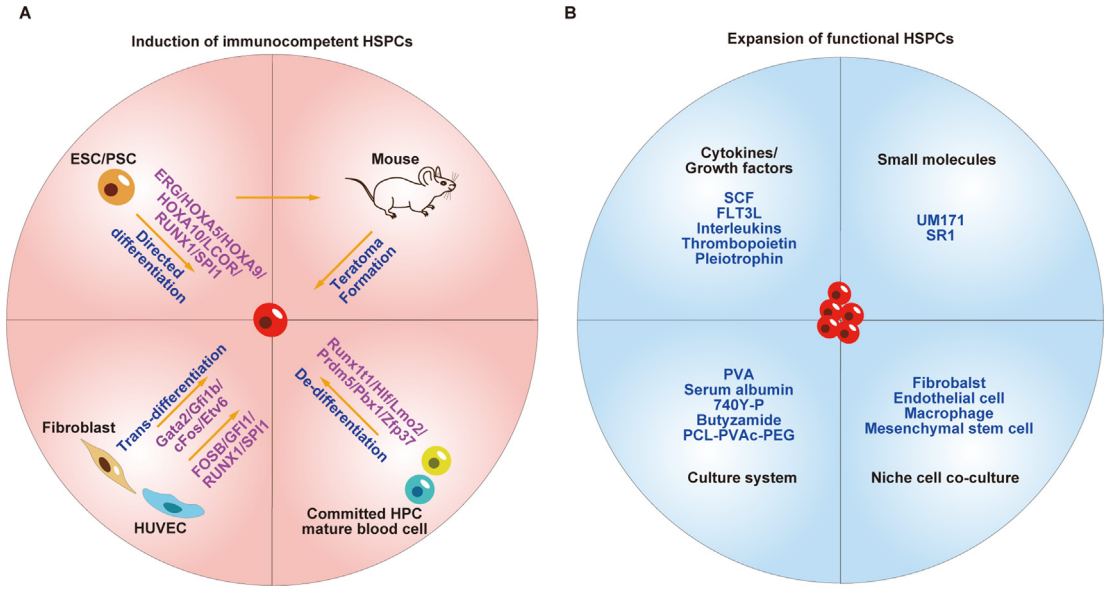

Fig. 3. The strategy of HSPC induction and expansion in vitro. **A**: HSPC induction via directed differentiation (under culture condition or within terotomas [Amabile et al., 2013]), trans-differentiation, and de-differentiation. **B**: HSPC expansion via manipulation of agonists/antagonists or optimization of culture systems. ESC, embryonic stem cell; PSC, pluripotent stem cell; HUVEC, human umbilical vein EC; HPC, hematopoietic progenitor cell; FLT3L, FMS-like tyrosine kinase 3 ligand; PVA, polyvinyl alcohol; PCL–PVAc–PEG, polyvinyl caprolactam–polyvinyl acetate–polyethylene glycol graft copolymer.

(2014) performed transiently ectopic expression of *Runx1t1*, *Hlf*, *Lmo2*, *Prdm5*, *Pbx1*, and *Zfp37* in mouse committed HPCs (e.g., myeloid and lymphoid progenitors) and mature myeloid cells. This manipulation reprograms these committed hematopoietic cells into HSC-like cells with multilineage repopulating and serial transplantation abilities; meanwhile, the gene expression profiles of the induced HSCs are similar to those of endogenous BM HSCs.

Efficient expansion of HSPCs

The current methods to efficiently expand HSPCs are to manipulate agonists/antagonists or optimize culture systems (Fig. 3B). Cytokines and growth factors are a representative type of agonists for HSPC expansion. For example, SCF, thrombopoietin, and interleukins (e.g., interleukin-11) are widely used to expand mouse HSPCs; SCF, thrombopoietin, interleukins (e.g., interleukin-6), FMS-related tyrosine kinase 3 ligand, and pleiotrophin serves as additives for human HSPC expansion (Wilkinson et al., 2020). By contrast, transforming growth factor-β and chemokine ligand have been shown to be antagonists for HSPC expansion (Csaszar et al., 2012). The removal of antagonists via medium changes or other methods is required for long-term expansion of HSPCs. Several newly identified small molecules have also been shown to be agonists for HSPC expansion. For example, the inhibition of aryl hydrocarbon receptor can promote the expansion of human HSPCs (Boitano et al., 2010; Rentas et al., 2016). StemRegenin 1 (SR1) is a purine derivative and has been demonstrated to be an antagonist of aryl hydrocarbon receptor. The treatment of SR1 leads to an about 17-fold expansion of human cord blood-derived HSCs with hematopoietic reconstitution ability in the immunodeficient mice (Boitano et al., 2010). UM171 is one of the pyrimidoindole derivatives and is able to promote human HSPC expansion for about 13-fold changes after 20-week culture (Fares et al., 2014). Mechanistically, UM171 preserves H3K4me2 and H3K27ac which are rapidly decreased during HSPC culture in vitro (Chagraoui et al., 2021). The optimization of culture systems is another strategy to achieve long-term expansion of HSPCs. The optimized culture system with the replacement of serum albumin by polyvinyl alcohol promotes mouse HSPC expansion for 236−898-fold changes (Wilkinson et al., 2019). Similarly, the optimized culture system with the replacement of endogenous cytokines and albumin by chemical agonists and a caprolactam-based polymer facilitates a long-term expansion (more than 30 days) and maintains the engraftment potential of human HSPCs (Sakurai et al., 2023).

Concluding remarks

In this Review, we first introduce the developmental events that successively occur along primitive hematopoiesis, pro-definitive hematopoiesis, and definitive hematopoiesis. In the primitive and pro-definitive hematopoiesis, there emerge primitive hematopoietic lineages and lineage-restricted HPCs. We then summarize the advances in the earliest hematopoietic specification event at the stage of mesodermal differentiation, with a focus on the developmental paths from mesoderm to primitive hematopoietic lineages and the master regulators-driven signaling cascades. We also discuss the debate on the contribution of EMPs to blood vasculatures in multiple extra- or intra-embryonic organs/tissues. In the definitive hematopoiesis, multipotent HSPCs are developed from a specialize arterial ECs, namely HECs. Given that the developing HSPCs are derived from different ECs/HECs via EHT, the molecular features and biological significances of heterogeneous ECs/HECs are discussed. One of the biological significances is that inter- and intra-organ/tissue EC/HEC heterogeneity is the earliest cause of HSPC heterogeneity. The nascent HSPCs are transported into a transient hematopoietic organ (i.e., fetal liver in mammals) via blood circulation. Although the fetal liver has long been considered to be an important site mainly supporting HSPC expansion, its diverse roles in supporting HSPC maturation, expansion, and differentiation have been unraveled in mice currently. In the section of postnatal hematopoiesis, we discuss the application of multi-modal omics to profile molecular landscapes of chromosomal organizations, chromatin states, epigenetic modifications, transcriptome features, and RNA epigenetics in the HSPCs from different postnatal stages or during HSPC differentiation. In addition, we also summarize recent advances in the niche regulation of BM maintenance of postnatal HSPCs. Finally, we discuss the revolutions of methodology guiding in vitro induction and expansion of HSPCs. Currently, the unresolved problems existed in the in vitro induction of HSPCs include time-consuming culture process, complicated genetic manipulation, and low efficiency of induction. Among them, the complicated genetic manipulation is likely to be improved via small molecules-mediated chemical reprogramming (Wang et al., 2023). In addition, whether there exist graft-versus-host disease and relapse post transplantation of cord blood HSPCs expanded in vitro awaits further investigation in clinical trials (Cohen et al., 2020). Future studies are needed to jointly employ live imaging and lineage tracing to record the fate transition process during developmental and postnatal hematopoiesis; to carefully apply different methods (non-invasive vs. invasive) to distinguish between cell fates and lineage potentials; to fully integrate multi-modal omics data to decipher fundamental principles underlying cell fate determinations; to construct hematopoietic organoids to recapitulate induction and expansion of *bona fide* HSPCs under native conditions. Promoting the experimental methods into clinical trials makes the basic research count.

Conflict of interest

The authors declare no conflict of interest.

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