HEMATOPOIETIC STEM CELLS

The genesis of human hematopoietic stem cells

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Developmental hematopoiesis consists of multiple, partially overlapping hematopoietic waves that generate the differentiated blood cells required for embryonic development while establishing a pool of undifferentiated hematopoietic stem cells (HSCs) for postnatal life. This multilayered design in which active hematopoiesis migrates through diverse extra and intraembryonic tissues has made it difficult to define a roadmap for generating HSCs vs non–self-renewing progenitors, especially in humans. Recent single-cell studies have helped in identifying the rare human HSCs at stages when functional assays are unsuitable for distinguishing them from progenitors. This approach has made it possible to track the origin of human HSCs to the unique type of arterial endothelium in the aorta-gonad-mesonephros region and document novel benchmarks for HSC migration and maturation in the conceptus. These studies have delivered new insights into the intricate process of HSC generation and provided tools to inform the in vitro efforts to replicate the physiological developmental journey from pluripotent stem cells via distinct mesodermal and endothelial intermediates to HSCs.

Introduction

Since human pluripotent stem cells (PSCs) were derived from blastocysts[1] and subsequently reprogrammed from fibroblasts,[2] major efforts have been put into the in vitro generation of blood and immune cells for regenerative or cancer therapies and disease modeling. However, better understanding of human HSC genesis is required for PSC differentiation into transplantable HSCs.[3-5] Mammalian developmental hematopoiesis occurs in multiple temporal waves and anatomical niches that generate both differentiated blood cells for the embryo and undifferentiated HSCs for life-long hematopoiesis[6-8] (Figure 1). How developing HSCs acquire multilineage differentiation and self-renewal ability remains unanswered. Decoding the intrinsic and extrinsic mechanisms guiding HSCs requires accurate definitions of HSC identity and location, which is challenging because of limited access to human developmental tissues and suboptimal assays to validate immature human HSCs.[9] Novel lineage-tracing, barcoding, and in vivo imaging tools have greatly advanced our understanding of mouse developmental hematopoiesis.[6,10] However, the discoveries from mice cannot be directly translated to human settings because of the different anatomy of extraembryonic tissues, pregnancy duration and timing of birth, and species-specific regulatory mechanisms[11,12] (Figure 1).

Developmental hematopoiesis was classically divided into 2 waves. The primitive hematopoietic wave, mainly derived from the extraembryonic yolk sac, generates the first blood cells in the precirculation conceptus and provides the embryo with oxygen, immune protection, and tissue-remodeling capabilities. The definitive wave, traditionally linked to the embryo, generates self-renewing HSCs that make differentiated blood cells for the lifetime. However, recent studies have shown that developmental hematopoiesis cannot be explained by only 2 waves and locations, given that several intermediate progenitors (often termed transient definitive or prodefinitive[6,7]) with properties between primitive hematopoietic cells and HSCs were discovered in both extraembryonic and intraembryonic tissues. These concepts have been summarized in several comprehensive reviews.[4,6,8,13,14] Because of the inconsistencies in the literature for the term "definitive" hematopoiesis (ie, anything not primitive or only HSC-generating), we refer to the true definitive hematopoietic wave as the "HSC-forming" wave. Because developing HSCs are intermixed with circulating HSC-independent progenitors, pinpointing their unique regulatory programs has been challenging.[4,7] Single-cell technologies have provided new tools to identify human HSCs based on their molecular signatures (Table 1[15-31]). In this review, we summarize how these studies have shaped our understanding of the genesis of human HSCs and identify areas for future studies to facilitate robust HSC generation in vitro.

The paradox of developmental hematopoiesis: blood formation before HSCs

Primitive hematopoiesis in extraembryonic tissues

The first blood-forming cells in mammalian embryos arise not from HSCs but from primitive hematopoietic progenitors.[32,33] These differentiation-primed progenitors originate from the precirculation yolk sac (YS) at Carnegie stages 7-8 (CS7-8) of human development[34] (16-18.5 days after fertilization; embryonic day 7-8 [E7-8] in mice) and generate primitive
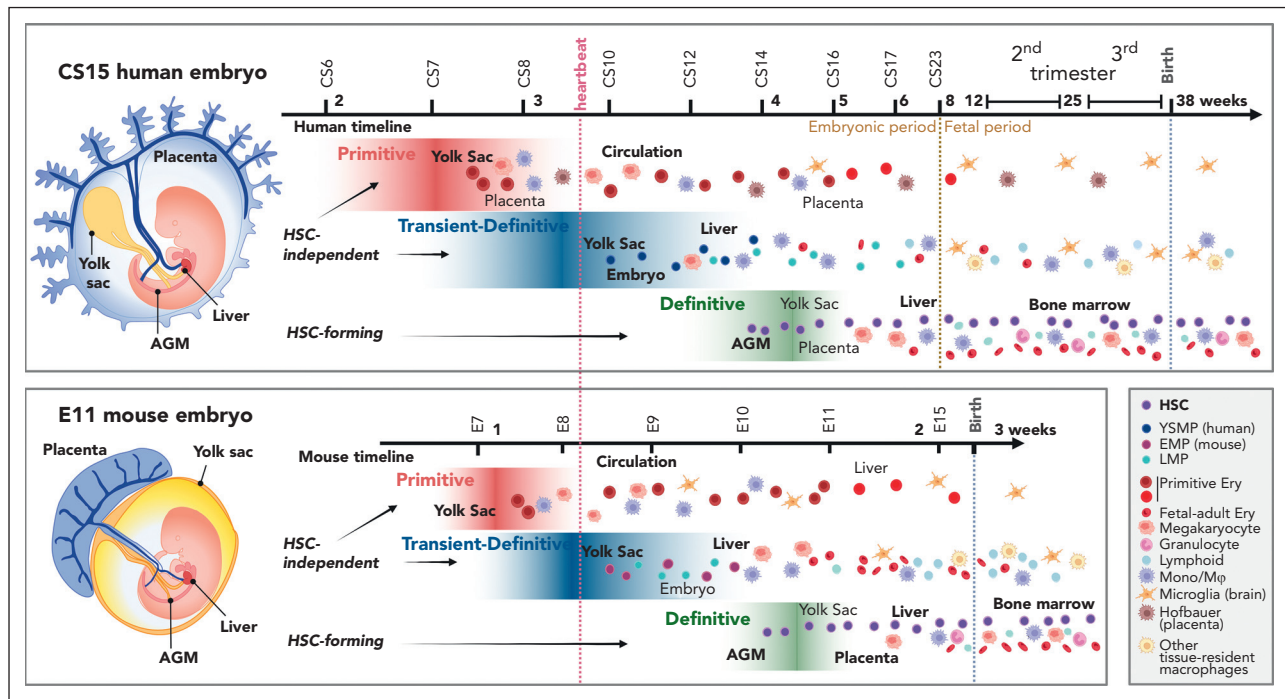
Figure 1. Comparative view of multilayered hematopoiesis during human and mouse development. Hematopoiesis is a conserved developmental process in mammals, but the anatomy of the hematopoietic sites, duration of the hematopoietic waves, and output of the progenitors differ between human and mouse. (Left) Human and mouse embryos are depicted at the stage when HSC emergence peaks (CS15; 5 weeks; E11). Although embryonic structures and the main vascular circuitry are similar, the anatomy of extraembryonic tissues is different. The human placenta is villous type and surrounds the embryo and amniotic membrane, whereas the yolk sac is a balloon-like appendage inside the amnion. The mouse placenta is labyrinthine-type, and the yolk sac surrounds the embryo and amnion. In both species, blood flows from the aorta through the vitelline and umbilical arteries to the yolk sac and the placenta and returns to the embryo through the liver via vitelline and umbilical veins. Human hematopoiesis starts by 2.5 weeks (CS7) in the yolk sac, with the first, primitive progenitor wave, during which the main products are nucleated primitive erythroblasts that enter circulation. In addition, the precirculation placenta generates macrophages (Hofbauer cells) that assist in primitive erythroblast enucleation in the placental villi. At 3.25 weeks (CS8-CS9) the second, transient- (or pro) definitive wave of human hematopoiesis is initiated in the yolk sac and possibly in the embryo proper as well. In human, the second wave starts with YSMPs, followed by LMPs of yet unknown origin. HSC-independent progenitors give rise to tissue-resident macrophages, such as microglia, Langerhans cells, and Kupffer cells that can last into adulthood and HSC-independent lymphoid populations. HSC-independent macrophages may have diverse origins, and most brain microglia generation in mice is linked to primitive rather than second wave progenitors. Many of second wave progenitors colonize the liver where they differentiate to blood and immune cells to support development. Between 4 and 6 weeks (CS14-CS16) a third, HSC-forming, definitive hematopoietic wave arises in the AGM region and produces nascent HSCs. HSCs first migrate through the placenta and yolk sac before they seed the liver (CS17). During these transitions HSCs undergo maturation, limited expansion, and some give rise to terminally differentiated progeny. HSCs start generating multilineage progeny already in the first trimester liver and move to the BM during the second trimester to sustain postnatal hematopoiesis. Mouse hematopoiesis is aligned according to comparable developmental stages as in human. Because mouse embryogenesis is compressed into a much shorter timeframe, a greater overlap of developmental events and hematopoietic populations is apparent. The end of mouse gestation (3 weeks) compares to the early fetal period (9 weeks) of human development. Some progenitor populations differ in their lineage output, such as mouse yolk sac transient definitive progenitors are highly primed for erythromyeloid differentiation (EMPs), whereas the corresponding yolk sac progenitors in human show myeloid skewing (YSMPs), and the first signs of liver erythropoiesis link to HSCs. Weeks are referred to as developmental age, (ie, weeks after fertilization, which is 2 weeks less than gestational weeks or weeks from the last menstrual cycle). Dotted lines depict developmental milestones, such as the onset of heartbeat, transition from embryonic to fetal period during human development, and birth, which occurs at very different developmental stages in mouse and human. The main hematopoietic cell types are described (bottom right). EMPs, erythromyeloid progenitors; Ery, erythroid cell; Mono/Mφ, monocyte/macrophage.

erythroblasts, megakaryocytes, and macrophages (Figure 1). Primitive hematopoiesis was first observed in early morphological studies of human embryos[35] and has recently been validated via single-cell RNA-sequencing (scRNA-seq) in CS7 conceptus.[36] Primitive erythroblasts are initially nucleated, larger than definitive erythroid cells, and express embryonic ζ/ε globins with higher oxygen carrying capacity.[37,38] They enter circulation when the heartbeat begins (21-23 days, CS10[39,40]). Upon reaching placental vasculature, primitive erythroblasts enucleate between 5 and 10 weeks by interacting with macrophages (Hofbauer cells) in placental villous stroma,[41] after which they are replaced by a new wave of enucleated fetal erythroid cells expressing α/γ globins, which differentiate in the liver. Human placental macrophages are generated in situ without input from circulating cells,[41,42] implying that multiple sources of precirculation macrophages contribute to human primitive hematopoiesis (Figure 1). The origin of macrophages in mouse placenta remains controversial.[43-45]

Transient definitive hematopoietic progenitor waves

Primitive hematopoiesis is followed by a second wave of HSC-independent progenitors (transient definitive or prodefinitive) that have partial multilineage differentiation capacity but do not differentiate at their place of origin[33,46] (Figure 1). In mice, the second wave is dominated by yolk sac–derived $CD41^+cKit^+CD16/32^+$ erythromyeloid progenitors that jump-start fetal liver (FL) erythropoiesis at approximately E10.5.[47] Recent studies also revealed lymphoid potential among HSC-independent progenitors, overturning the dogma that lymphoid potential only associates with HSCs.[48-52]

Lineage-tracing studies also linked yolk sac HSC–independent hematopoietic progenitors to long-lived progeny, including brain microglia and other tissue-resident macrophages,[53-55] and unique lymphoid populations[56] that persist through adulthood and participate in neurogenerative diseases[57] and

Table 1. Human HSC ontogeny and characteristics

Human hematopoietic cell type	Description and functional properties	RNA expression signature	Protein markers	References
HSC endothelial precursors				
AGM AE CS13/15 wk 4 to wk 5	Arterial ECs from the AGM region lining the aorta	RUNX1⁻HOXA9⁺MEIS2⁺SOX17⁺	CDH5⁺CD34⁺CD90⁺CXCR4⁺GJA5⁺CD43⁻CD45⁻	15,16
AGM pre-HE CS13/15 wk 4 to wk 5	Subset of AE cells from the aorta preceding the activation of hematopoietic transition	RUNX1⁻HOXA9⁺MEIS2⁺SOX17⁺IL-33⁺ALDH1A1⁺DKK1⁺	CDH5⁺CD34⁺CD90⁺CXCR4⁺GJA5⁺IL-33⁺ALDH1A1⁺CD43⁻CD45⁻	15
AGM HE CS13/15 wk 4 to wk 5	Subset of AE on the ventral side of dorsal aorta undergoing EHT (RUNX1 expression without SPN/PTPRC activation)	RUNX1⁺HOXA9⁺MEIS2⁺SOX17⁺ALDH1A1⁺DKK1⁺	CDH5⁺CD34⁺CD90⁺CD44⁺KCNK17⁺CD43⁻CD45⁻	15,16
HSC				
AGM HSCs CS14/15 wk 4 to wk 5	HSC emerging via IAHC, undifferentiated state with immature endothelial/megakaryocytic surface features and displaying functional immaturity. Rare highly potent transplantable HSCs.	RUNX1⁺MLLT3⁺HOXA9⁺SPINK2⁺HLF⁺MECOM⁺LIN28B⁺IGFBP2⁺HOXB9⁺	CD34⁺CD90⁺KCNK17⁺CD43⁺CD45⁺CDH5⁺EMCN⁺ACE⁺PROCR⁺	15,17,18
Yolk sac HSCs CS14/CS16	HSCs displaying functional immaturity but diminished endothelial features compared with AGM HSCs. Transplantable HSCs found at CS16. Erythroid priming and transcriptional similarity to CS17 liver HSCs observed.	RUNX1⁺MLLT3⁺HOXA9⁺SPINK2⁺HLF⁺MECOM⁺LIN28B⁺IGFBP2⁺HOXB9⁺	CD34⁺CD90⁺CD43⁺CD45⁺CDH5⁺/⁻ACE⁺PROCR⁺	15,17,19
Placental HSCs CS14 to wk 9	HSCs displaying functional immaturity but diminished endothelial features compared with AGM HSCs. Erythroid priming and transcriptional similarity to CS17 liver HSCs are observed. Presence of GPI80⁺ HSPCs observed at 5 wk.	RUNX1⁺MLLT3⁺HOXA9⁺SPINK2⁺HLF⁺MECOM⁺LIN28B⁺IGFBP2⁺HOXB9⁺	CD34⁺CD90⁺CD43⁺CD45⁺GPI80⁺CDH5⁺/⁻ACE⁺PROCR⁺	15,19,20
Embryonic liver HSC CS17/20 wk 6 to wk 7	HSC displaying functional immaturity but showing suppression of endothelial features. Erythro-megakaryocyte-myeloid differentiation-primed.	RUNX1⁺MLLT3⁺HOXA9⁺SPINK2⁺HLF⁺MECOM⁺IGFBP2⁺LIN28B⁺HOXB9⁺	CD34⁺CD90⁺CD43⁺CD45⁺GPI80⁺ACE⁺PROCR⁺	15,17,19
First trimester fetal liver HSC from wk 8 to wk 12	Transplantable HSCs, progressive decline of fetal programs, gradual acquisition of multipotency and quiescence, and increased expression of MHC class I and II and PROM1	RUNX1⁺MLLT3⁺HOXA9⁺SPINK2⁺HLF⁺MECOM⁺HEMGN	CD34⁺CD90⁺CD43⁺CD45⁺GPI80⁺ACE⁺PROCR⁺PROM1⁺HLA-DR⁺	15,19,21
Second trimester liver HSCs from wk 13 to wk 20	Transplantable HSCs, gradual loss of fetal properties and transition to quiescence, and robust expression of MHC class I and II and PROM1	RUNX1⁺MLLT3⁺HOXA9⁺SPINK2⁺HLF⁺MECOM⁺HEMGN⁺MSI2ʰⁱ⁺	CD34⁺CD90⁺CD43⁺CD45⁺GPI80⁺ACE⁺PROCR⁺PROM1⁺HLA-DR⁺	15,21-26
Second trimester fetal BM HSCs	Transplantable HSCs, further transition to quiescence, and robust expression of MHC class I and II and PROM1	RUNX1⁺MLLT3⁺HOXA9⁺SPINK2⁺HLF⁺MECOM⁺HEMGN⁺MSI2ʰⁱ	CD34⁺CD90⁺CD43⁺CD45⁺GPI80⁺ACE⁺PROCR⁺PROM1⁺HLA-DR⁺	20,23,25-27
Cord blood HSCs	Transplantable HSCs, complete acquisition of functional maturation signature, and robust expression of MHC class I and II	RUNX1⁺MLLT3⁺HOXA9⁺SPINK2⁺HLF⁺MECOM⁺HEMGN⁺MSI2ʰⁱ	CD34⁺CD90⁺CD43⁺CD45⁺GPI80⁻ACE⁺PROCR⁺PROM1⁺HLA-DR⁺SELL⁺EMCN⁺CD164⁺	15,28-31

Characteristics of different human HSC endothelial precursors and HSC maturation stages.

neoplasia,[58,59] respectively (Figure 1). These populations may have several cellular origins in the yolk sac. In mice, most tissue-resident macrophages arise from Kitl-dependent second wave progenitors, whereas brain microglia originate from Kitl-independent progenitors.[60]

The second hematopoietic wave has been less well-defined in human embryos. scRNA-seq analysis helped identify early human yolk sac–derived myeloid progenitors (YSMPs) that seed the liver by CS12,[61] likely representing human transient definitive hematopoiesis. Comparison of CS11 YSMPs with nascent HSCs (identified by the presence of HSC signature RUNX1+HOXA9+MLLT3+MECOM+HLF+SPINK2+)[15] using scRNA-seq showed that although YSMPs express many genes associated with HSCs (HLF, RUNX1, and SPINK2), they lack HSC hallmarks (medial HOXA genes and robust MLLT3 and MECOM expression[28,62,63]) and express embryonic signature genes (LIN28A).[15] scRNA-seq analysis of CS10 embryo also helped detect intra-embryonic hematopoietic progenitors that are similar to CS11 YSMPs and linked to the embryonic hemogenic endothelium (HE), which is distinct from HSC-forming HE in CS13 to CS15 aorta-gonad-mesonephros (AGM).[15,16] The mouse embryo can generate HSC-independent lympho-myeloid progenitors (LMPs) and multipotent progenitors (MPPs) that partially overlap with HSC generation,[49,52,64-66] but it is unclear whether there is a mouse counterpart for human CS10 HE observed at the onset of the heartbeat. The compressed developmental timeline in mice[67] likely leads to greater overlap between progenitor- and HSC-forming hemogenic waves (Figure 1).

B- and T-lymphoid potential, previously considered a proxy for the HSC lineage in in vitro assays, has been linked to HSC-independent progenitors in both mouse and human embryos.[16,32,52,61,68] scRNA-seq of CS14 human conceptus identified a SPINK2+–interleukin–7 receptor–positive (SPINK2+IL-7+) LMPs that are distinct from early CS10 to CS11 myeloid progenitors and from CS13 to CS15 HSCs. At CS14, they are found mainly in the liver, head, and heart, suggesting that they enter circulation and colonize the liver before HSCs. The CS14 liver SPINK2+ progenitors lack the expression of HSC signature genes that regulate HSC fate (HLF, HOXA9, MLLT3, and MECOM) (Table 2[15,16,62,63,69-96]) and associate with lymphoid (IL-7R; EBF1) and macrophage (C1QA, MRC, and CX3CR1) trajectories in CS14-17 livers.[15] Similar lymphoid-biased IL-7R+CD7hi progenitor was reported in CS15 liver in another independent study.[61] Between weeks 7 and 9, several lymphoid populations appear in the liver, including thymus-seeding progenitors and progenitors for B-cells and innate lymphoid cells.[61,97,98] Further investigations are needed to discriminate between HSC-dependent or HSC-independent origin for the various immune progenitors. Nevertheless, differentiation trajectories suggest that HSC-derived lymphopoiesis begins in the liver by the end of embryonic period (8 weeks), as HSC-independent SPINK2+IL-7R+ LMPs disappear.[15]

scRNA-seq of other human developmental tissues has revealed unexpected locations and differentiation trajectories of hematopoiesis, including erythropoiesis in the skin and kidney during first trimester and hematopoietic stem and progenitor cell (HSPC) formation in the lung during second trimester.[16,22,23,99] Future studies will elucidate the origin and role of these hematopoietic populations.

Developmental journey of HSCs
Generation of immature HSCs in the AGM region
The third, definitive hematopoiesis wave generates HSCs, but their anatomical and cellular origin has been difficult to pinpoint. The AGM is a functionally validated primary site of HSC emergence in mice and humans, whereas the contribution of extraembryonic tissues is still under investigation. Human HSCs emerge at CS14 to CS16 (4-5 weeks) on the ventral side of the dorsal aorta via intra-aortic hematopoietic clusters (IAHCs)[40] (Figures 2 and 3[15]; Table 1), which were first observed in early histological studies.[100] Hematopoietic potential of CD34+ cells from CS13 to CS14 AGM vasculature and IAHCs was established using long-term culture–initiating cell stroma coculture,[40] but there was no proof whether they represent HSCs or progenitors. HSC quantification using the gold-standard human HSC functional assay and transplantation to immunodeficient mice showed rare transplantable HSCs in human AGM (1 per embryo),[17] similar to that in mice,[101,102] suggesting that most cells in IAHCs are HSC-independent progenitors or immature HSCs that cannot repopulate adult bone marrow (BM). Nonetheless, the presence of rare cells with extensive regenerative potential in immunodeficient mice confirmed the intrinsic potency of AGM HSCs.[17,18] Single-cell studies in mouse embryos identified a molecularly distinct HSC population that is much larger than those detected using transplantation assays, supporting the hypothesis that AGM HSCs are functionally immature.[103] This conclusion was validated by lineage-tracing and barcoding studies[10] and optimized in vitro maturation cultures that derived transplantable mouse HSCs from immature pre-HSCs and endothelial precursors.[66,98,104,105]

To track the emergence and maturation of HSCs in human embryos in which in vivo interrogations are not possible and functional maturation in vitro has not been achieved,[18] scRNA-seq analysis was used to distinguish human HSCs from their differentiated progeny and HSC-independent progenitors throughout ontogeny. This distinction could be done using a 6 gene HSC signature that consists of transcription factors governing definitive HE or HSC specification (RUNX1 and HOXA9) or maintenance (MLLT3, MECOM, and HLF) (Table 2), and HSC or progenitor gene SPINK2.[15] CS14-15 AGM harbored a molecularly uniform population of hundreds of nascent HSCs, supporting the concept that human AGM HSCs are more numerous than those detected using transplantation assay and functionally more immature.

HSC maturation in the fetal liver
The 6-gene HSC signature helped track the transition of HSCs between the AGM and liver approximately at CS17 (6 developmental weeks), after liver hematopoiesis was already established (Figure 2; Table 1). The liver is the main site for active hematopoiesis throughout the first and second trimesters and a "melting pot" for the different hematopoietic waves. The liver rudiment emerges at approximately CS10 (week 3) and becomes populated first by primitive erythroid cells, macrophages, and circulating progenitors. Immunostaining and in vitro studies suggested that the CS12 liver is seeded by CD34+CD45+ hematopoietic progenitors,[40] which single-cell studies identified as myeloid-primed YSMPs[61] (Figure 1). An early landmark study uncovered multiple lineages of

Table 2. Transcriptional regulation of human HSC specification and maintenance

Human HSC transcriptional regulators	Role in hematopoiesis	Functional validation	References
HSC specification			
SCL/TAL1	Promotes HE establishment and suppresses cardiac fate during mesoderm specification. Augments hematopoietic specification in hPSC cultures.	Mouse KO, mESC KO differentiation, and hESC OE model	69,70-72
SOX17	Essential for establishment of arterial identity, definitive HE specification, and EHT. Marks definitive HE during hPSC differentiation.	Mouse KO, mESC KO differentiation, hESC reporters, and loss of function/OE models.	63,73-76
Medial HOXA genes	HOXA5, 7, and 9 are landmarks of specification to definitive hematopoietic fate in vitro and in vivo, HOXA9 associates with HSC proliferation and HOXA7 with suppressing primitive hematopoiesis associated programs in human fetal HSCs	Mouse KO and human FL-HSC KD/OE	15,62,63,74,77-80
RUNX1	Necessary for IAHC formation in both HSC-independent and HSC-forming EHT. Indicates HE specification during human development and in hPSC cultures.	Mouse KO, mESC differentiation, and hESC reporters	15,63,73,81
MYB	Required during definitive hematopoiesis for HSC self-renewal and suppression of differentiation programs	Mouse KO and hESC reporters	16,82,83
GFI1/GFI1B	Promote hematopoietic identity by suppressing endothelial program and inducing quiescence	Mouse KO and small molecule inhibitors during hESC differentiation	84-87
HSC maintenance			
MLLT3	Essential for human HSC self-renewal. Sustains HSC transcriptional network via DOT1L. Expressed from pre-HE stage onwards. Levels increase during human HSC maturation. Expands functional HSCs.	KD and OE in human FL HSCs and CB-HSCs	15,88
HLF	Defines undifferentiated HSPC and promotes HSC quiescence. Highly specific for undifferentiated HSPC but expressed also in some second wave progenitors in human, although not observed in mouse erythromyeloid progenitors. Essential for human HSC function.	Mouse KOs, mouse reporters, and human CB HSC reporters. KD in human FL HSCs	15,88-92
MECOM	Maintains HSC proliferation in a dosage-dependent manner. Expressed highly in EC throughout EHT and HSCs. High expression levels define LT-HSC lineage. Essential for human HSC function.	Mouse KOs, mouse reporters. KD in human FL HSCs	15,88,91,93,94
MSI2	Promotes human HSC expansion through AHR signaling inhibition	Mouse KO, human HSC KD/OE	95,96

Key transcription factors functionally implicated in human HSC development in vivo or in vitro and their major roles are indicated.

AHR, aryl hydrocarbon receptor; CB, cord blood; ESC, embryonic stem cells; h, human; KD, knockdown; KO, knockout; m, murine; OE, overexpression.

hematopoietic cells in the liver at CS13/CS14.[106] These likely represent circulating primitive erythroid cells and myeloid or LMPs and their progeny, because the liver is colonized by molecularly defined HSCs only at 6 weeks[15] and transplantable HSCs only after weeks 7 or 8[17,18,21,107] (Table 1). These observations imply that human liver hematopoiesis, like that in mice, is sequentially initiated by HSC-independent progenitors, followed by immature HSCs.

Previous studies identified surface markers to isolate human embryonic (<8 weeks) and fetal HSCs, including angiotensin I converting enzyme (ACE) (from AGM to CB),[19,28,107] GPI-80 (VNN2, from placenta to fetal BM),[20,24] and EPCR (PROCR, all stages including cultured CB).[24,108,109] However, little has been known about the process how nascent human HSCs acquire functional maturity and robust BM engraftment ability. scRNA-seq analysis of HLF+ human HSCs throughout development uncovered gene expression changes, reflecting HSC

maturation[15] (Figure 2). Although HSC identity is evident upon HSC emergence based on the expression of known HSC transcription factors, their maturation to transplantable HSCs involves temporal changes in transcriptional programs, reflecting unique surface phenotypes, biological processes, and behaviors. Moreover, their differentiation trajectories evolve from predominantly erythro-megakaryocytic during the first trimester to myelo-lymphoid and multilineage output in the second trimester.[15,22] Concomitantly, they suppress endothelial and megakaryocytic surface features and embryonic or early fetal intrinsic programs (LIN28B, IGFBP2, and posterior HOXB genes) and proliferation genes, increase the expression of HSC stemness regulators (MLLT3, HLF, and MSI2), and acquire maturity markers PROM1/CD133 and major histocompatibility complex (MHC) class II molecules (Figure 2; Table 1). Mouse studies revealed that HSCs also functionally require the expression of MHC class I and II genes.[110,111] Together, the fetal HSC identity marker and newly identified maturation surface markers facilitate
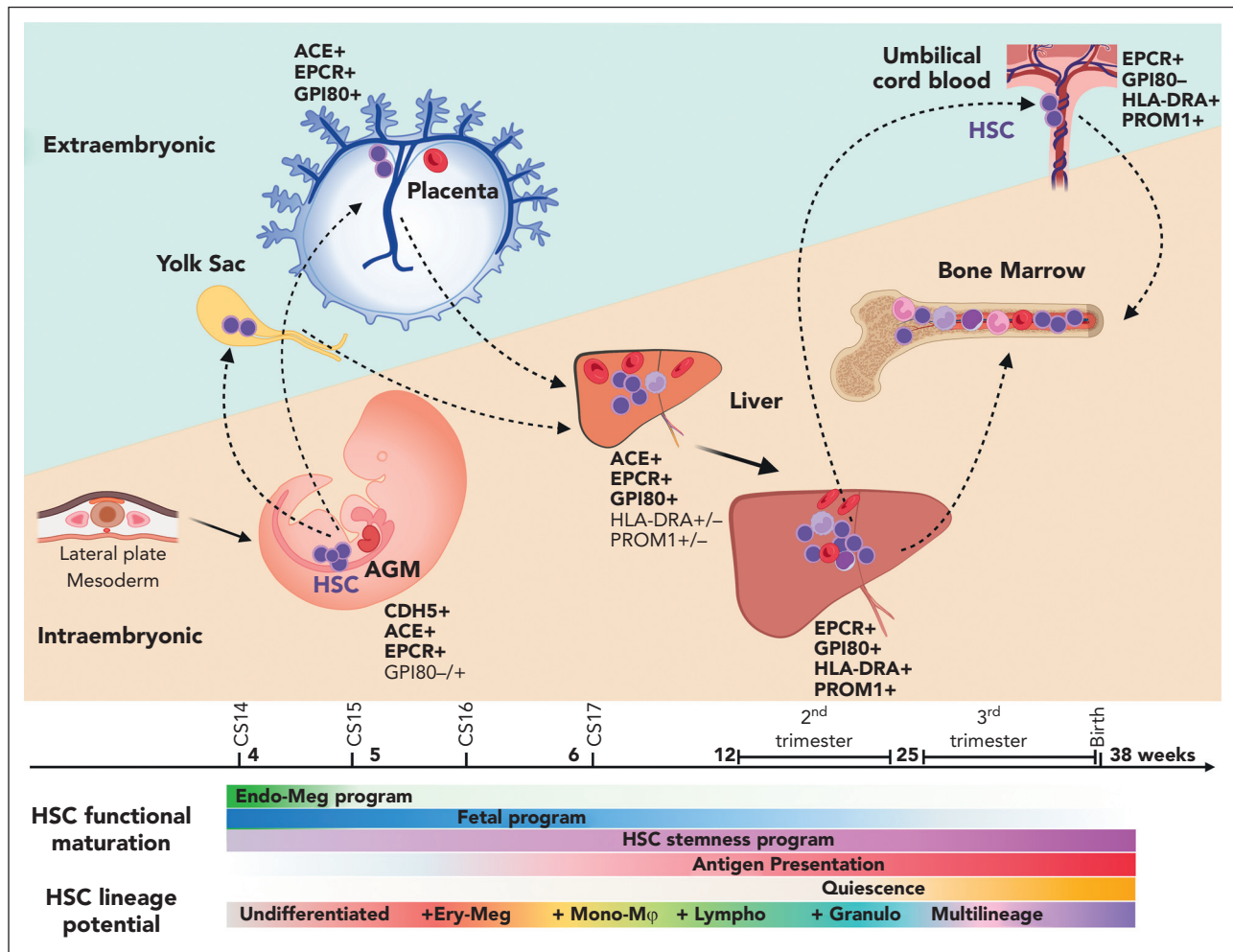
Figure 2. The developmental journey of human HSCs. Schematic diagram showing human HSCs throughout gestation as they migrate between intra and extraembryonic niches. HSCs are generated in the lateral plate mesoderm–derived AGM region, where they show an undifferentiated and developmentally immature phenotype. As HSCs emerge from the IAHCs, they follow circulation to the placenta and the yolk through the main arterial outlets of the aorta, the umbilical and vitelline arteries. In extraembryonic sites, HSCs suppress endothelial identity and show transcriptional priming for erythro-megakaryocytic fates. HSCs return to the embryo after CS17 as they colonize the liver for developmental maturation and initiate lineage differentiation. Over time, HSCs acquire multilineage differentiation ability as they switch from erythro-megakaryocytic bias to multilineage potential, including myeloid and lymphoid lineages. They express fetal HSC surface marker GPI80 and acquire HSC maturity markers (PROM1/CD133 and antigen presentation machinery). They gradually lose intrinsic fetal properties and boost HSC self-renewal program, while transitioning toward homeostatic state. During the second trimester, HSCs transition to the BM, where they transition to deeper quiescence and complete developmental maturation. HSC shuttling between intra- and extraembryonic sites (dashed arrows) continues throughout gestation as evidenced by their presence in the placental umbilical cord blood at birth.

evaluating the human fetal HSC maturity stage via flow cytometry[15] (Table 1; Figure 2).

The dogma that fetal liver is the site for HSC expansion has been recently questioned using mice. Previous studies evaluated HSC expansion based on the increase in engraftable HSCs during development.[112] However, confetti-based lineage tracing implied that HSCs with life-long potential do not expand massively in the liver, and the immunophenotypic HSCs that proliferate robustly between E12.5 and E14.5 are prone to differentiation rather than symmetric self-renewal.[113] This suggests that the increase in transplantable HSCs in mouse liver is largely explained by functional maturation rather than extensive HSC expansion. In humans, scRNA-seq analysis of HLF[+] fetal liver HSCs over developmental time evidenced declining expression of proliferation genes at the end of the embryonic period, implying a shift toward homeostatic HSC state already during the first trimester.[15]

HSCs in extraembryonic tissues preceding liver colonization

In mice, HSCs were found in extraembryonic yolk sac, placenta, and vitelline and umbilical arteries, and hematopoiesis was also reported in the head and the heart.[41,114-121] The contribution of these sites to human developmental hematopoiesis remains unresolved. Transplantable HSCs have been verified in the human yolk sac at CS16 (5.5 weeks) before that in the liver (7-8 weeks).[17,21] In the placenta, transplantable HSCs were found at week 9 onwards[121] and in the second trimester, in another study,[17] but HSPCs with fetal HSC phenotype (CD34^{+}CD38^{lo}CD90^{+}GPI80^{+}) that expand in HSC cultures were detected at week 5 onwards.[20] These discrepancies may be caused by HSC immaturity or technical factors, such as tissue dissociation methods or pregnancy termination using antiprogestins that target placental vasculature. scRNA-seq of CS14 (4.5 weeks) conceptus helped detect similar populations of molecularly defined nascent HSCs in the AGM,
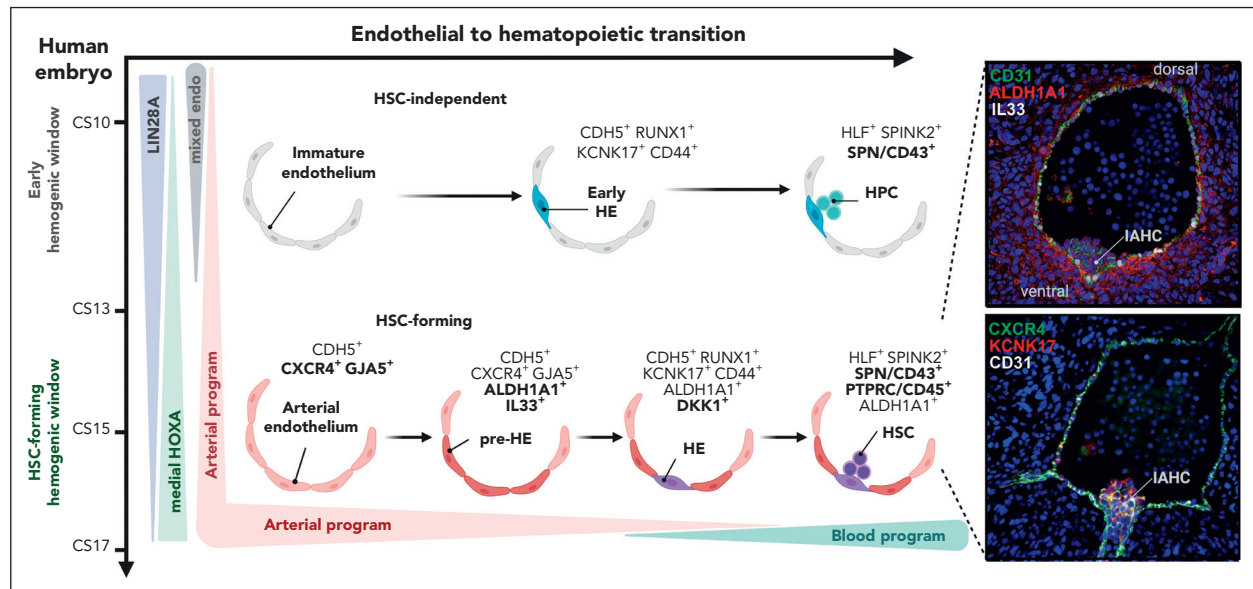
CALVANESE and MIKKOLA

Figure 3. Specification and emergence of HSCs via EHT in the human AGM region. Schematic representation of the establishment of HSC-forming HE in the human AGM region. At CS14/15, an arterially specified HOXA-patterned EC gives rise to an arterial subset termed pre-HE, characterized by ALDH1A1 (RA-signaling) and IL-33 expression. HE is specified from pre-HE by the hematopoietic transcription factor RUNX1 in cells that upregulate KCNK17, CD44, and WNT inhibitors (DKK1), and gives rise to SPINK2+CD45+ nascent HSCs. In the earlier embryo (CS10-11), HE that generates HSC-independent progenitors is specified from immature endothelial precursors that express embryonic genes (LIN28A) and lack HOXA patterning and arterial identity. (Right) Immunofluorescence validation of stage-specific markers indicate the emergence of human HSCs from IAHC in sections of CS15 human AGM. Modified from Calvanese et al.[15]

placenta, and yolk sac before HSCs appeared in the liver, heart, or head.[15] Molecular signatures suggested that extraembryonic HSC–like cells are 1 step away from the most immature AGM HSCs, which exhibit highest expression of endothelial genes and minimal connections to differentiating cells. Surprisingly, extraembryonic HSCs at CS14 are more similar to the first molecularly defined HSCs in the liver 1 or 2 weeks later (CS17) than AGM HSCs in the same CS14 conceptus. Because the liver is directly downstream to the yolk sac and the placenta in the circulatory route, the extraembryonic HSCs are likely on track to colonize the liver.[15,61] Despite lacking engraftment ability, CS17 liver HSCs are HOXA-patterned and linked to HOXA+ multilineage progeny, including the fetal erythroid cells, implying that they belong to the HSC lineage. Whether the first HSCs colonizing the liver contribute equally to life-long hematopoiesis than their slightly later counterparts or whether they merely support fetal hematopoiesis is not known. Evidence for gradually increasing HSC potency was found in mouse placenta and livers between E11.5/12.5 and E12.5/13.5, respectively.[115] These data further suggest that extensive tendency for proliferation and differentiation in immature HSCs before homeostatic programs are established may negatively correlate with sustained HSC properties.

HSC transition to fetal BM

Onset of human BM hematopoiesis begins with myeloid cell colonization of the cavity of long bones, followed by vascularization at approximately week 8.[122] Single-cell and transplantation studies detected long-term engraftable HSCs at approximately week 12,[27] and fully active BM hematopoiesis was evident by week 14.[27,122] Expression of fetal HSC marker GPI80 was confirmed in the second trimester (15-18 weeks) fetal BM

HSCs.[20] Fetal BM HSCs have been associated with unique properties, including a differentiation bias to B-cell and granulocytic lineages. Direct comparison of human FL and BM HSC/MPP populations between 17 and 22 weeks using scRNA-seq suggested a shift to a more quiescent state upon transition to fetal BM,[25] although this was based on HSC/MPP population as a whole rather than molecularly defined HSCs. Future studies are needed to define how the niche changes between human FL and BM to support HSC maturation and life-long maintenance.

Distinct temporal contributions of HSC-independent progenitors and HSCs to developmental hematopoiesis in mice and humans

Lineage-tracing studies in mice have proven instrumental for dissecting the contributions of various developmental populations to pre- and postnatal hematopoiesis. Flt3-Cre marking helped identify a unique, developmentally restricted liver HSPC population that is absent in postnatal tissues[123] but can acquire multilineage engraftment ability upon transplantation. During normal development, their contribution to adult HSCs is minimal, but they can generate innate-like lymphoid cells. A great extent of mouse postnatal lymphopoiesis was also tracked to Flt3-Cre–marked, embryo-derived MPPs, which were postulated to originate from immature HSC precursors, pre-HSCs.[48] In contrast, studies on Hlf- and Evi1-reporter mice implied that long-term HSCs, marked by high Evi1/Mecom expression in Hlf+ cells, only contribute significantly to hematopoiesis after birth.[93] Lineage tracing using Lyve1-Cre indicated a shift between progenitor-driven erythropoiesis and HSC-driven erythropoiesis during late mouse gestation, which was based on Lyve1-marking that matched yolk sac progenitors vs adult HSCs.[124]

Although mouse-human comparisons using similar lineage-tracing techniques are not possible, scRNA-seq trajectories in human developmental hematopoietic tissues indicate much greater contribution of transcriptomically defined HSCs to human developmental hematopoiesis than that in mouse tissues. Human embryonic liver at 6 weeks harbors newly colonized HOXA+ HSC that directly connects to similarly patterned erythroid, megakaryocytic, and myeloid cells.[15] By week 8, the differentiation trajectories include HSC-derived EBF1+IL-7R+ B-lymphoid cells, which replace HSC-independent SPINK2+IL-7R+ LMPs in the liver at earlier stage. These studies suggest that HSCs contribute substantially to hematopoiesis throughout human gestation, beginning from late embryonic stages (6-8 weeks).[15,22,23,25,26] Although these relationships are difficult to confirm during native human developmental hematopoiesis, new tools have been developed to demonstrate clonal relationships, such as mitochondrial DNA mutations and could potentially be used to track human developmental hematopoiesis.[125] Nonetheless, the differences between HSC-dependent vs HSC-independent progenitor contributions to mouse and human developmental and postnatal hematopoiesis are profound and may be explained by the longer human gestation (38 weeks vs 3 weeks in mice) and the earlier birth in mice that corresponds to beginning of fetal period (week 9) of human development (Figure 1). These differences should be considered when using the mouse to model human hematological diseases that develop in utero, such as infant leukemias and trisomy 21–associated preleukemic disorder.[59]

Unique properties of HSC-forming arterial HE

Emergence of human HSCs from arterial endothelium

The cellular origin of HSCs has been difficult to demonstrate because of the intermixing of populations via circulation. Analysis of heartbeat-deficient Ncx1−/− mouse embryos documented multilineage de novo hematopoiesis in the AGM, placenta, and yolk sac, but assaying in vivo HSC potential was hampered by early lethality.[117,126-128] Several in vivo and in vitro live-imaging and lineage-tracing models have since documented direct emergence of hematopoietic cells from endothelial precursors via endothelial-to-hematopoietic transition (EHT).[129-133] Nevertheless, the identity and location of the unique type of HE that generates HSCs has been poorly defined.

scRNA-seq studies on mouse embryos first documented a link between nascent HSCs and aortic HE[64,68] and were followed by studies on human embryos[15,16,134] (Figure 3). HSC-primed HE in the human AGM region could be identified based on the coexpression of endothelial markers (CDH5 and SOX7) and HSPC transcription factors (RUNX1 and MYB) in cells that have not yet induced hematopoietic surface markers (PTPRC/CD45 and SPN/CD43). Decreased expression of arterial marker CXCR4 and induction of CD44 pinpointed the arterial subset undergoing EHT.[16] Single-cell analysis of CS14/15 AGM tissues uncovered a direct molecular trajectory from HOXA-patterned arterial endothelial cells (ECs) to nascent AGM HSCs via unique arterial endothelial subset, termed pre-HE, which induced IL-33 and ALDH1A1 expression. The decline of IL-33

and the induction of landmarks for HE (KCNK17) and HSPC (SPINK2) marked the progression of EHT (Table 1; Figure 3).[15] Spatial transcriptomics helped confirm IL-33, ALDH1A1, KNCK17, and SPINK2 as indicators of human HSC genesis and confirmed this process in IAHCs on the ventral side of the dorsal aorta.[15]

Hematopoietic progenitors in the CS14/15 AGM or liver that lack HOXA patterning did not show molecular connection to the aortic endothelium in scRNA-seq analyses at this stage, indicating a different origin.[15] Nevertheless, HE could already be detected in human embryos at CS10[16] (Figure 3). This early HE associated with embryonic hematopoietic cells that were transcriptomically more similar to HSC-independent progenitors in the CS11 yolk sac than to nascent HSCs in the CS13/15 AGM.[15] Although both intra and extraembryonic early (CS10-11) hematopoietic cells shared many HSC-associated genes (HLF and SPINK2) and linked to RUNX1+KCNK17+ HE, they expressed unique embryonic genes (LIN28A) and lacked HSC hallmarks (HOXA patterning and HSC stemness regulators MLLT3 and MECOM). In addition, CS10 embryonic HE did not evidence strong arterial identity or hallmarks of pre-HE (IL-33 and ALDH1A1). The HSC-forming HE in CS14/15 AGM was pinpointed as the crossroads between essential arterial endothelial and hematopoietic signaling networks. Although Notch, Wnt, and transforming growth factor β pathways are highly active in arterial EC and pre-HE, attenuation of these pathways at the HE stage implies that their timely suppression may be necessary for HSC emergence.[15]

These single-cell level molecular observations revealed that although HE generation and EHT can occur in multiple stages and anatomical sites, the outcome of EHT depends on regional patterning (eg, HOXA gene expression characteristic for the AGM region) and the signaling environment where the HE was specified. Activation of hemogenic program in EC without arterial identity generates HSC-independent progenitors. Shifts among these hemogenic programs in the embryo begin at approximately CS11, as the first signs of pre-HE marker IL-33 transcript appear. HSC-forming hemogenic window in the aorta was defined to occur from CS13 to 15,[15] after which no molecular connection between AGM arterial endothelium and HSCs could be established,[134] implying tight temporal control. Nevertheless, microdissection of CS16 AGM followed by transcriptomics identified a secreted niche factor endothelin 1 (EDN1), expressed ventrally in the aorta near IAHC, which enhances both mouse and human hematopoiesis.[134]

These studies illustrate the power of single-cell and spatial technologies in pinpointing the cellular origin and intermediates generating human HSCs and defining the environment required for HSC genesis and the timing for signaling switches that occur during EHT. The findings in human embryos concur with studies in mice, in which clonal assays and single-cell transcriptomics concluded that HSCs emerge from Cxcr4+ arterially specified HE, whereas most blood and immune progenitors are derived from Cxcr4− HE.[66] To verify clonal relationships between endothelial populations in human embryos and transplantable HSCs using functional assays, it will be important to optimize culture conditions for human HE and nascent HSCs to promote the acquisition of complete HSC potential.

Recapitulating human HSC and progenitor waves from PSCs

Modeling of human developmental hematopoietic waves in vitro

Directed differentiation of human embryonic stem cells and human induced PSCs to HSCs has been improved over the years using many different strategies.[3-5] Small molecules and unique PSC reporter lines for hematopoietic regulators have helped in recapitulating and monitoring the early steps of human developmental hematopoiesis that direct mesoderm to distinct hematopoietic waves (Table 2; Figure 4).[4] However, generation of long-term reconstituting human HSCs that can function in xenotransplantation has not been achieved solely by directed differentiation but requires viral transduction of HSC regulators and is still very inefficient.[77] Nevertheless, transcription factor–driven lineage reprogramming provides a proof-of-concept for HSC generation in vitro.[4] Because comprehensive reviews for both approaches are available,[3-5,135,136] we focus on novel insights that scRNA-seq studies have provided toward producing HSCs from human PSCs.

Major progress toward this goal was achieved using morphogens, including activin A, BMP4, and FGF2, and small molecule agonists and antagonists to direct PSC differentiation to distinct embryonic mesodermal precursors that generate AGM- vs YS-type HE (reviewed previously[4]) (Figure 4). Stimulation of activin A signaling 1 or 2 days after mesoderm induction generates $KDR^+CD235a/b^+$ mesoderm that produces YS-like primitive erythroid cells and macrophages, followed by MPPs that generate γ/δ T-lymphoid cells.[137-139] Although multipotent, these YS-like precursors are distinct from the HSC lineage. scRNA-seq analysis helped identify comparable populations in extraembryonic mesoderm of CS7 conceptus.[36,137] Inhibition of activin/nodal signaling in the same window promoted the generation of $KDR^+CD235a/b^-$ mesoderm,[139] the precursor for HE that generates AGM-type hematopoietic cells with T-lymphoid potential.[73] The expression of medial HOXA genes (HOXA5-9) reflects patterning to definitive, AGM-like hematopoiesis[62,63] and is essential for HSC function.[25,62] Timed stimulation of retinoic acid (RA) signaling, a key player in AGM-like hematopoiesis in mice,[140] could induce HOXA gene expression and other programs critical for HSC formation.[62,141] NOTCH activation in PSC-derived HSPC helped specify aorta-like HE that produces multipotent progenitors,[142] acting through SOX17-mediated CDX2 induction and orchestration of HOXA genes and arterial programs.[74] Despite these advances, transplantable HSCs were not generated in these studies.
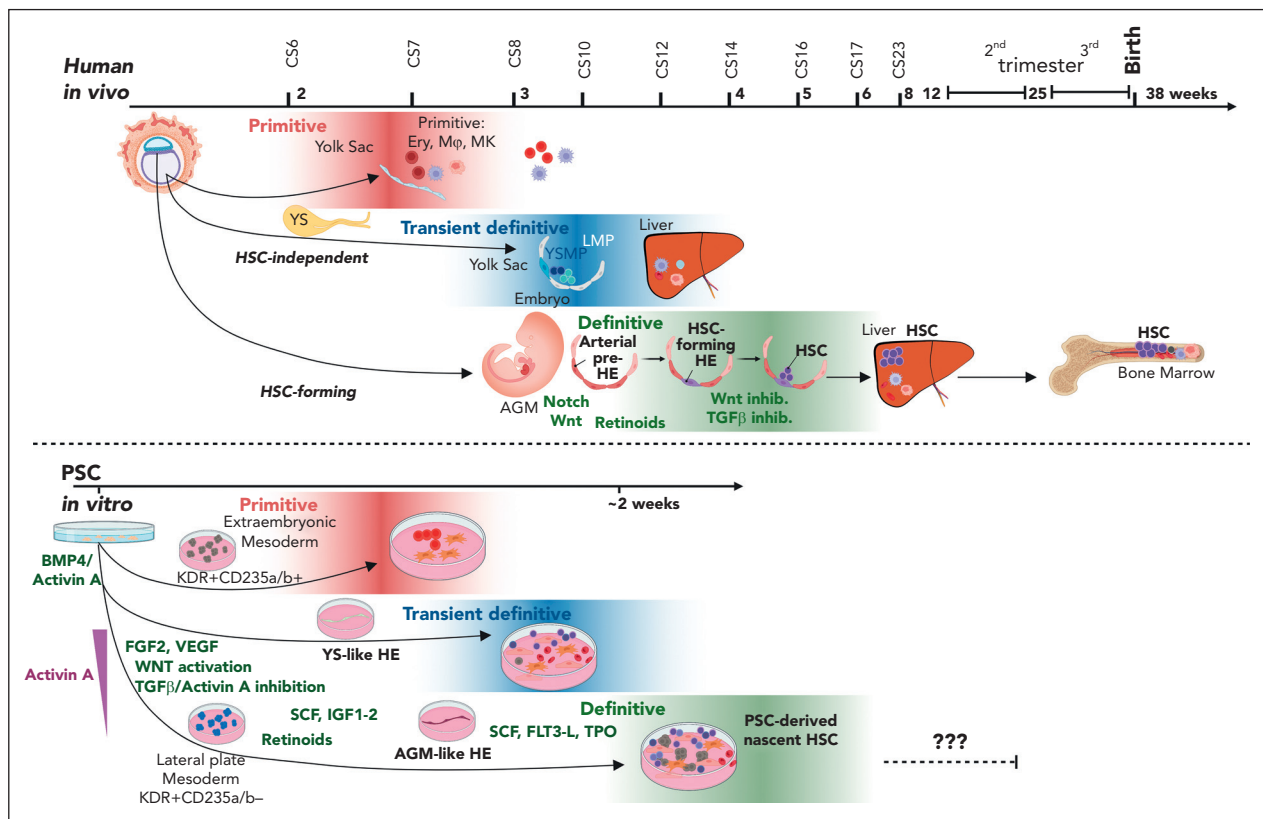

Figure 4. Comparative view of human developmental hematopoiesis in human embryo in vivo and during PSC differentiation in vitro. Top schematic diagram represents developmental hematopoietic waves in humans. The primitive wave composed of primitive erythroid, megakaryocytic, and macrophage progenitors is followed by a transient definitive wave consisting of multiple hematopoietic progenitors (YSPMs and LMPs; Figure 1). These progenitor waves also give rise to long-lasting tissue-resident macrophages and unique γ/δ T-cells. At CS14/CS16, the definitive hematopoietic wave in the embryo gives rise to HSC-forming HE and the HSC lineage. An arterially specified EC in the aorta, characterized by active Notch, WNT, and transforming growth factor βsignaling and patterned by retinoids, undergoes EHT by inhibiting WNT and transforming growth factor β pathways and activating hematopoietic signaling. HSC functional maturation is attained in the liver during following weeks via yet unknown niche signals. It has been possible to recapitulate the early steps of the distinct hematopoietic waves to differentiate PSCs to both YS-like and AGM-like HE precursors and their progeny (bottom). BMP4-mediated mesoderm induction with sequential addition of other morphogens and cytokines, including small molecule–mediated WNT activation, activin A inhibition, and use of retinoids, have been optimized for the generation of diverse intra and extraembryonic-type mesodermal precursors. However, the generation of functionally mature and robustly engraftable human HSCs in culture requires further optimization of the culture microenvironment. SCF, stem cell factor; TPO, thrombopoietin; VEGF, vascular endothelial growth factor.

Identification of maturation blocks in PSC-derived, AGM-like HSCs using single-cell maps of human developmental hematopoiesis

Single-cell transcriptomics has facilitated the direct comparison of in vitro generated HE and hematopoietic cells to in vivo counterparts from distinct developmental tissues and stages. Comprehensive single-cell maps of human developmental hematopoiesis and immune cell development spanning the early hematopoietic waves in the yolk sac and embryo proper through fetal and neonatal stages[15,23,97] are now available and can be used to determine developmental milestones and roadblocks in differentiating PSC to transplantable HSCs or to specific immune cells for cancer immunotherapies. Single-cell analysis of HE and HSPCs generated by combining early WNT stimulation with retinoid agonists revealed that dependance on these pathways distinguishes different mesodermal and HE populations. Although Wnt-dependence categorized intraembryonic mesoderm from extraembryonic mesoderm, RA-dependence subdivided the intraembryonic HE into 2 distinct subsets. RA-dependent HE resembled the HSC-forming HE in CS13 human embryos the most.[97,141] Use of artificial intelligence trained using 7- or 17-week old liver HSC/MPPs[22] identified a small, transient population of FL-HSC–like cells among human PSC–derived hematopoietic progeny.[143] This also revealed higher NOTCH signaling and lower mitochondrial oxidative phosphorylation in in vivo HSPCs, providing evidence that the direct comparison via single-cell transcriptomics can help pinpoint limitations of current PSC differentiation protocols. Neural network–based label transfer method helped compare PSC-HSPCs using in vivo reference map spanning human developmental hematopoiesis and validate PSC differentiation of human induced PSCs to AGM- and placental-like nascent HSCs found in CS14/15 conceptus.[15] However, the PSC-derived HSCs were unable to mature to fetal liver HSC stages,[15] uncovering a distinct developmental block in differentiation. Stage-specific scorecards illustrating human HSC developmental stages further validated this conclusion.[15] A similar approach was used in a PSC differentiation study that used DLL4 and VCAM1 to stimulate NOTCH signaling during EHT, followed by successful T-cell differentiation. Although comparison of the in vitro generated HSPCs to fetal liver scRNA-seq data suggested similarity to FL HSCs, comparison with the scRNA-seq map that also includes early human hemogenic tissues helped verify successful specification to nascent AGM-like HSCs but showed little evidence of maturation to liver stages.[144]

Lack of HSC developmental maturation of PSC-HSPCs to liver stages is not surprising because most protocols have focused on specification of AGM-like HE. Future efforts will be needed to dissect the signals for HSC functional maturation in FL and BM niches and apply these findings in culture. As the failure of PSC-derived tissue stem cells to mature beyond embryonic stages is observed also with other systems,[145,146] overcoming these developmental blocks is a major unsolved problem. Because maturation of human HSCs may require prolonged culture, which in itself is detrimental for human HSCs,[147] recent advances in human HSC maintenance or expansion involving unique small molecules (SR1 and UM171),[148,149] chemical agonists substituting cytokines,[150] or distinct niche cells[151] and materials[152,153] may help improve the HSC maturation niche. More permissive in vivo models for functional validation of immature human HSC will also be needed. Ability to direct human developmental hematopoiesis in vitro is crucial for establishing disease-in-a-dish-models for inherited diseases, such as hemoglobinopathies or infant leukemias. The new single-cell technologies that can evaluate epigenetic changes, splice isoforms, surface protein expression, and spatial location will provide a lens through which these important developmental events can be studied in humans.

Authorship

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REFERENCES

1. Thomson JA, Itskovitz-Eldor J, Shapiro SS, et al. Embryonic stem cell lines derived from human blastocysts. Science. 1998;282(5391):1145-1147.

2. Takahashi K, Tanabe K, Ohnuki M, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell. 2007;131(5):861-872.

3. Ditadi A, Sturgeon CM, Keller G. A view of human haematopoietic development from the Petri dish. Nat Rev Mol Cell Biol. 2017;18(1):56-67.

4. Ivanovs A, Rybtsov S, Ng ES, Stanley EG, Elefanty AG, Medvinsky A. Human haematopoietic stem cell development: from the embryo to the dish. Development. 2017;144(13):2323-2337.

5. Ding J, Li Y, Larochelle A. De novo generation of human hematopoietic stem cells from pluripotent stem cells for cellular therapy. Cells. 2023;12(2):321.

6. Vink CS, Mariani SA, Dzierzak E. Embryonic origins of the hematopoietic system: hierarchies and heterogeneity. HemaSphere. 2022;6(6):e737.

7. Canu G, Ruhrberg C. First blood: the endothelial origins of hematopoietic progenitors. *Angiogenesis*. 2021;24(2):199-211.

8. Dzierzak E, Bigas A. Blood development: hematopoietic stem cell dependence and independence. *Cell Stem Cell*. 2018;22(5):639-651.

9. Goyama S, Wunderlich M, Mulloy JC. Xenograft models for normal and malignant stem cells. *Blood*. 2015;125(17):2630-2640.

10. Ganuza M, Clements W, McKinney-Freeman S. Specification of hematopoietic stem cells in mammalian embryos: a rare or frequent event? *Blood*. 2022;140(4):309-320.

11. Parekh C, Crooks GM. Critical differences in hematopoiesis and lymphoid development between humans and mice. *J Clin Immunol*. 2013;33(4):711-715.

12. Pishesha N, Thiru P, Shi J, Eng JC, Sankaran VG, Lodish HF. Transcriptional divergence and conservation of human and mouse erythropoiesis. *Proc Natl Acad Sci U S A*. 2014;111(11):4103-4108.

13. Ciau-Uitz A, Monteiro R, Kirmizitas A, Patient R. Patient R. Developmental hematopoiesis: ontogeny, genetic programming and conservation. *Exp Hematol*. 2014;42(8):669-683.

14. Kumar A, D'Souza SS, Thakur AS. Understanding the journey of human hematopoietic stem cell development. *Stem Cells Int*. 2019;2019:2141475.

15. Calvanese V, Capellera-Garcia S, Ma F, et al. Mapping human haematopoietic stem cells from haemogenic endothelium to birth. *Nature*. 2022;604(7906):534-540.

16. Zeng Y, He J, Bai Z, et al. Tracing the first hematopoietic stem cell generation in human embryo by single-cell RNA sequencing. *Cell Res*. 2019;29(11):881-894.

17. Ivanovs A, Rybtsov S, Welch L, Anderson RA, Turner ML, Medvinsky A. Highly potent human hematopoietic stem cells first emerge in the intraembryonic aorta-gonad-mesonephros region. *J Exp Med*. 2011;208(12):2417-2427.

18. Ivanovs A, Rybtsov S, Anderson RA, Medvinsky A. Vast self-renewal potential of human AGM region HSCs dramatically declines in the umbilical cord blood. *Stem Cell Reports*. 2020;15(4):811-816.

19. Zhang Y, Clay D, Mitjavila-Garcia MT, et al. VE-cadherin and ACE Co-expression marks highly proliferative hematopoietic stem cells in human embryonic liver. *Stem Cells Dev*. 2019;28(3):165-185.

20. Prashad SL, Calvanese V, Yao CY, et al. GPI-80 defines self-renewal ability in hematopoietic stem cells during human development. *Cell Stem Cell*. 2015;16(1):80-87.

21. Oberlin E, Fleury M, Clay D, et al. VE-cadherin expression allows identification of a new class of hematopoietic stem cells within human embryonic liver. *Blood*. 2010;116(22):4444-4455.

22. Popescu D-M, Botting RA, Stephenson E, et al. Decoding human fetal liver haematopoiesis. *Nature*. 2019;574(7778):365-371.

23. Suo C, Dann E, Goh I, et al. Mapping the developing human immune system across organs. *Science*. 2022;376(6597):eabo0510.

24. Vanuytsel K, Villacorta-Martin C, Lindstrom-Vautrin J, et al. Multi-modal profiling of human fetal liver hematopoietic stem cells reveals the molecular signature of engraftment. *Nat Commun*. 2022;13(1):1103.

25. Ranzoni AM, Tangherloni A, Berest I, et al. Integrative single-cell RNA-Seq and ATAC-Seq analysis of human developmental hematopoiesis. *Cell Stem Cell*. 2021;28(3):472-487.e7.

26. Jardine L, Webb S, Goh I, et al. Blood and immune development in human fetal bone marrow and Down syndrome. *Nature*. 2021;598(7880):327-331.

27. Zheng Z, He H, Tang XT, et al. Uncovering the emergence of HSCs in the human fetal bone marrow by single-cell RNA-seq analysis. *Cell Stem Cell*. 2022;29(11):1562-1579.e7.

28. Jokubaitis VJ, Sinka L, Driessen R, et al. Angiotensin-converting enzyme (CD143) marks hematopoietic stem cells in human embryonic, fetal, and adult hematopoietic tissues. *Blood*. 2008;111(8):4055-4063.

29. Pellin D, Loperfido M, Baricordi C, et al. A comprehensive single cell transcriptional landscape of human hematopoietic progenitors. *Nat Commun*. 2019;10(1):2395.

30. Velten L, Haas SF, Raffel S, et al. Human haematopoietic stem cell lineage commitment is a continuous process. *Nat Cell Biol*. 2017;19(4):271-281.

31. Zheng S, Papalexi E, Butler A, Stephenson W, Satija R. Molecular transitions in early progenitors during human cord blood hematopoiesis. *Mol Syst Biol*. 2018;14(3):e8041.

32. Ghosn E, Yoshimoto M, Nakauchi H, Weissman IL, Herzenberg LA. Hematopoietic stem cell-independent hematopoiesis and the origins of innate-like B lymphocytes. *Development*. 2019;146(15):dev170571.

33. Palis J. Hematopoietic stem cell-independent hematopoiesis: emergence of erythroid, megakaryocyte, and myeloid potential in the mammalian embryo. *FEBS Lett*. 2016;590(22):3965-3974.

34. Luckett WP. Origin and differentiation of the yolk sac and extraembryonic mesoderm in presomite human and rhesus monkey embryos. *Am J Anat*. 1978;152(1):59-97.

35. Bloom W, Bartelmez GW. Hematopoiesis in young human embryos. *Am J Anat*. 1940;67(1):21-53.

36. Tyser RCV, Mahammadov E, Nakanoh S, Vallier L, Scialdone A, Srinivas S. Single-cell transcriptomic characterization of a gastrulating human embryo. *Nature*. 2021;600(7888):285-289.

37. Manning JM, Manning LR, Dumoulin A, Padovan JC, Chait B. Embryonic and fetal human hemoglobins: structures, oxygen binding, and physiological roles. In: Hoeger U, Harris JR, eds. *Vertebrate and Invertebrate Respiratory Proteins, Lipoproteins and Other Body Fluid Proteins*. Springer International Publishing; 2020:275-296.

38. Peschle C, Migliaccio AR, Migliaccio G, et al. Embryonic—fetal Hb switch in humans: studies on erythroid bursts generated by embryonic progenitors from yolk sac and liver. *Proc Natl Acad Sci U S A*. 1984;81(8):2416-2420.

39. Hikspoors JPJM, Kruepunga N, Mommen GMC, Köhler SE, Anderson RH, Lamers WH. A pictorial account of the human embryonic heart between 3.5 and 8 weeks of development. *Commun Biol*. 2022;5(1):226.

40. Tavian M, Hallais MF, Peault B. Emergence of intraembryonic hematopoietic precursors in the pre-liver human embryo. *Development*. 1999;126(4):793-803.

41. Van Handel B, Prashad SL, Hassanzadeh-Kiabi N, et al. The first trimester human placenta is a site for terminal maturation of primitive erythroid cells. *Blood*. 2010;116(17):3321-3330.

42. Thomas JR, Appios A, Calderbank EF, et al. Primitive haematopoiesis in the human placenta gives rise to macrophages with epigenetically silenced HLA-DR. *Nat Commun*. 2023;14:1764.

43. Chen X, Tang AT, Tober J, et al. Mouse placenta fetal macrophages arise from endothelial cells outside the placenta. *Dev Cell*. 2022;57(23):2652-2660.e3.

44. Liang G, Zhou C, Jiang X, et al. De novo generation of macrophage from placenta-derived hemogenic endothelium. *Dev Cell*. 2021;56(14):2121-2133.e6.

45. Liang G, Liu F. Response to matters arising: characterization of placental fetal macrophages. *Dev Cell*. 2022;57(23):2601-2603.

46. Soares-da-Silva F, Freyer L, Elsaid R, et al. Yolk sac, but not hematopoietic stem cell-derived progenitors, sustain erythropoiesis throughout murine embryonic life. *J Exp Med*. 2021;218(4):e20201729.

47. McGrath KE, Frame JM, Fegan KH, et al. Distinct sources of hematopoietic progenitors emerge before HSCs and provide functional blood cells in the mammalian embryo. *Cell Rep*. 2015;11(12):1892-1904.

48. Patel SH, Christodoulou C, Weinreb C, et al. Lifelong multilineage contribution by embryonic-born blood progenitors. *Nature*. 2022;606(7915):747-753.

49. Zhu Q, Gao P, Tober J, et al. Developmental trajectory of prehematopoietic stem cell formation from endothelium. *Blood.* 2020; 136(7):845-856.

50. Böiers C, Carrelha J, Lutteropp M, et al. Lymphomyeloid contribution of an immune-restricted progenitor emerging prior to definitive hematopoietic stem cells. *Cell Stem Cell.* 2013;13(5):535-548.

51. Lin Y, Yoder MC, Yoshimoto M. Lymphoid progenitor emergence in the murine embryo and yolk sac precedes stem cell detection. *Stem Cells Dev.* 2014;23(11): 1168-1177.

52. Hadland B, Yoshimoto M. Many layers of embryonic hematopoiesis: new insights into B-cell ontogeny and the origin of hematopoietic stem cells. *Exp Hematol.* 2018;60:1-9.

53. Ginhoux F, Greter M, Leboeuf M, et al. Fate mapping analysis reveals that adult microglia derive from primitive macrophages. *Science.* 2010;330(6005):841-845.

54. Gomez Perdiguero E, Klapproth K, Schulz C, et al. Tissue-resident macrophages originate from yolk-sac-derived erythro-myeloid progenitors. *Nature.* 2015;518(7540): 547-551.

55. Menassa DA, Muntslag TAO, Martin-Estebane M, et al. The spatiotemporal dynamics of microglia across the human lifespan. *Dev Cell.* 2022;57(17):2127-2139. e6.

56. Gentek R, Ghigo C, Hoeffel G, et al. Epidermal γδ T cells originate from yolk sac hematopoiesis and clonally self-renew in the adult. *J Exp Med.* 2018;215(12):2994-3005.

57. Mass E, Jacome-Galarza CE, Blank T, et al. A somatic mutation in erythro-myeloid progenitors causes neurodegenerative disease. *Nature.* 2017;549(7672):389-393.

58. Daniels J, Doukas PG, Escala MEM, et al. Cellular origins and genetic landscape of cutaneous gamma delta T cell lymphomas. *Nat Commun.* 2020;11(1):1806.

59. Mendoza-Castrejon J, Magee JA. Layered immunity and layered leukemogenicity: developmentally restricted mechanisms of pediatric leukemia initiation. *Immunol Rev.* 2023;315(1):197-215.

60. Azzoni E, Frontera V, McGrath KE, et al. Kit ligand has a critical role in mouse yolk sac and aorta–gonad–mesonephros hematopoiesis. *EMBO Rep.* 2018;19(10): e45477.

61. Bian Z, Gong Y, Huang T, et al. Deciphering human macrophage development at single-cell resolution. *Nature.* 2020; 582(7813):571-576.

62. Dou DR, Calvanese V, Sierra MI, et al. Medial HOXA genes demarcate haematopoietic stem cell fate during human development. *Nat Cell Biol.* 2016;18(6): 595-606.

63. Ng ES, Azzola L, Bruveris FF, et al. Differentiation of human embryonic stem cells to HOXA(+) hemogenic vasculature that resembles the aorta-gonad-mesonephros. *Nat Biotechnol.* 2016;34(11): 1168-1179.

64. Baron CS, Kester L, Klaus A, et al. Single-cell transcriptomics reveal the dynamic of haematopoietic stem cell production in the aorta. *Nat Commun.* 2018;9(1):2517.

65. Hou S, Li Z, Zheng X, et al. Embryonic endothelial evolution towards first hematopoietic stem cells revealed by single-cell transcriptomic and functional analyses. *Cell Res.* 2020;30(5):376-392.

66. Dignum T, Varnum-Finney B, Srivatsan SR, et al. Multipotent progenitors and hematopoietic stem cells arise independently from hemogenic endothelium in the mouse embryo. *Cell Rep.* 2021;36(11):109675.

67. Xue L, Cai J-Y, Ma J, et al. Global expression profiling reveals genetic programs underlying the developmental divergence between mouse and human embryogenesis. *BMC Genomics.* 2013;14(1):568.

68. Zhou F, Li X, Wang W, et al. Tracing haematopoietic stem cell formation at single-cell resolution. *Nature.* 2016; 533(7604):487-492.

69. Van Handel B, Montel-Hagen A, Sasidharan R, et al. Scl represses cardiomyogenesis in prospective hemogenic endothelium and endocardium. *Cell.* 2012;150(3):590-605.

70. Org T, Duan D, Ferrari R, et al. Scl binds to primed enhancers in mesoderm to regulate hematopoietic and cardiac fate divergence. *EMBO J.* 2015;34(6):759-777.

71. Lancrin C, Sroczynska P, Stephenson C, Allen T, Kouskoff V, Lacaud G. The haemangioblast generates haematopoietic cells through a haemogenic endothelium stage. *Nature.* 2009;457(7231):892-895.

72. Real PJ, Ligero G, Ayllon V, et al. SCL/TAL1 regulates hematopoietic specification from human embryonic stem cells. *Mol Ther.* 2012;20(7):1443-1453.

73. Ditadi A, Sturgeon CM, Tober J, et al. Human definitive haemogenic endothelium and arterial vascular endothelium represent distinct lineages. *Nat Cell Biol.* 2015;17(5): 580-591.

74. Jung HS, Uenishi G, Park MA, et al. SOX17 integrates HOXA and arterial programs in hemogenic endothelium to drive definitive lympho-myeloid hematopoiesis. *Cell Rep.* 2021;34(7):108758.

75. Clarke RL, Yzaguirre AD, Yashiro-Ohtani Y, et al. The expression of Sox17 identifies and regulates haemogenic endothelium. *Nat Cell Biol.* 2013;15(5):502-510.

76. Nakajima-Takagi Y, Osawa M, Oshima M, et al. Role of SOX17 in hematopoietic development from human embryonic stem cells. *Blood.* 2013;121(3):447-458.

77. Sugimura R, Jha DK, Han A, et al. Haematopoietic stem and progenitor cells from human pluripotent stem cells. *Nature.* 2017;545(7655):432-438.

78. Doulatov S, Vo LT, Chou SS, et al. Induction of multipotential hematopoietic progenitors from human pluripotent stem cells via respecification of lineage-restricted precursors. *Cell Stem Cell.* 2013;13(4): 459-470.

79. Lebert-Ghali CE, Fournier M, Dickson GJ, Thompson A, Sauvageau G, Bijl JJ. HoxA cluster is haploinsufficient for activity of hematopoietic stem and progenitor cells. *Exp Hematol.* 2010;38(11):1074-1086.e1-5. e1071-1075.

80. Ramos-Mejía V, Navarro-Montero O, Ayllón V, et al. HOXA9 promotes hematopoietic commitment of human embryonic stem cells. *Blood.* 2014;124(20): 3065-3075.

81. Chen MJ, Yokomizo T, Zeigler BM, Dzierzak E, Speck NA. Runx1 is required for the endothelial to haematopoietic cell transition but not thereafter. *Nature.* 2009; 457(7231):887-891.

82. White JR, Weston K. Myb is required for self-renewal in a model system of early hematopoiesis. *Oncogene.* 2000;19(9): 1196-1205.

83. Shah Z, Filonenko ES, Ramensky V, et al. MYB bi-allelic targeting abrogates primitive clonogenic progenitors while the emergence of primitive blood cells is not affected. *Haematologica.* 2021;106(8): 2191-2202.

84. Lancrin C, Mazan M, Stefanska M, et al. GFI1 and GFI1B control the loss of endothelial identity of hemogenic endothelium during hematopoietic commitment. *Blood.* 2012; 120(2):314-322.

85. Zeng H, Yücel R, Kosan C, Klein-Hitpass L, Möröy T. Transcription factor Gfi1 regulates self-renewal and engraftment of hematopoietic stem cells. *EMBO J.* 2004; 23(20):4116-4125.

86. Hock H, Hamblen MJ, Rooke HM, et al. Gfi-1 restricts proliferation and preserves functional integrity of haematopoietic stem cells. *Nature.* 2004;431(7011):1002-1007.

87. Thambyrajah R, Mazan M, Patel R, et al. GFI1 proteins orchestrate the emergence of haematopoietic stem cells through recruitment of LSD1. *Nat Cell Biol.* 2016; 18(1):21-32.

88. Calvanese V, Nguyen AT, Bolan TJ, et al. MLLT3 governs human haematopoietic stem-cell self-renewal and engraftment. *Nature.* 2019;576(7786):281-286.

89. Gazit R, Garrison BS, Rao TN, et al. Transcriptome analysis identifies regulators of hematopoietic stem and progenitor cells. *Stem Cell Reports.* 2013;1(3):266-280.

90. Lehnertz B, Chagraoui J, MacRae T, et al. HLF expression defines the human hematopoietic stem cell state. *Blood*. 2021; 138(25):2642-2654.

91. Yokomizo T, Watanabe N, Umemoto T, et al. Hlf marks the developmental pathway for hematopoietic stem cells but not for erythro-myeloid progenitors. *J Exp Med*. 2019; 216(7):1599-1614.

92. Komorowska K, Doyle A, Wahlestedt M, et al. Hepatic leukemia factor maintains quiescence of hematopoietic stem cells and protects the stem cell pool during regeneration. *Cell Rep*. 2017;21(12): 3514-3523.

93. Yokomizo T, Ideue T, Morino-Koga S, et al. Independent origins of fetal liver haematopoietic stem and progenitor cells. *Nature*. 2022;609(7928):779-784.

94. Goyama S, Yamamoto G, Shimabe M, et al. Evi-1 is a critical regulator for hematopoietic stem cells and transformed leukemic cells. *Cell Stem Cell*. 2008;3(2):207-220.

95. Park S-M, Deering RP, Lu Y, et al. Musashi-2 controls cell fate, lineage bias, and TGF-β signaling in HSCs. *J Exp Med*. 2014;211(1): 71-87.

96. Rentas S, Holzapfel N, Belew MS, et al. Musashi-2 attenuates AHR signalling to expand human haematopoietic stem cells. *Nature*. 2016;532(7600):508-511.

97. Zeng Y, Liu C, Gong Y, et al. Single-cell RNA sequencing resolves spatiotemporal development of pre-thymic lymphoid progenitors and thymus organogenesis in human embryos. *Immunity*. 2019;51(5): 930-948.e6.

98. Hadland B, Varnum-Finney B, Dozono S, et al. Engineering a niche supporting hematopoietic stem cell development using integrated single-cell transcriptomics. *Nat Commun*. 2022;13(1):1584.

99. Yeung AK, Villacorta-Martin C, Lindstrom-Vautrin J, et al. De-novo hematopoiesis from the fetal lung. *bioRxiv*. Preprint posted online 14 February 2022. doi: 2022.2002. 2014.480402

100. Minot CS. The origin of the angioblast and the development of the blood. *Manual Human Embryology*. 1912;2:498-534.

101. Medvinsky A, Dzierzak E. Definitive hematopoiesis is autonomously initiated by the AGM region. *Cell*. 1996;86(6):897-906.

102. Müller AM, Medvinsky A, Strouboulis J, Grosveld F, Dzierzak E. Development of hematopoietic stem cell activity in the mouse embryo. *Immunity*. 1994;1(4): 291-301.

103. Boisset JC, Clapes T, Klaus A, et al. Progressive maturation toward hematopoietic stem cells in the mouse embryo aorta. *Blood*. 2015;125(3):465-469.

104. Hadland BK, Varnum-Finney B, Poulos MG, et al. Endothelium and NOTCH specify and amplify aorta-gonad-mesonephros-derived hematopoietic stem cells. *J Clin Invest*. 2015;125(5):2032-2045.

105. Rybtsov S, Ivanovs A, Zhao S, Medvinsky A. Concealed expansion of immature precursors underpins acute burst of adult HSC activity in foetal liver. *Development*. 2016;143(8):1284-1289.

106. Migliaccio G, Migliaccio AR, Petti S, et al. Human embryonic hemopoiesis. Kinetics of progenitors and precursors underlying the yolk sac—liver transition. *J Clin Invest*. 1986; 78(1):51-60.

107. Zhang Y, Clay D, Mitjavila-Garcia MT, et al. VE-cadherin and ACE co-expression marks highly proliferative hematopoietic stem cells in human embryonic liver. *Stem Cells Dev*. 2019;28(3):165-185.

108. Subramaniam A, Talkhoncheh MS, Magnusson M, Larsson J. Endothelial protein C receptor (EPCR) expression marks human fetal liver hematopoietic stem cells. *Haematologica*. 2019;104(2):e47-e50.

109. Fares I, Chagraoui J, Lehnertz B, et al. EPCR expression marks UM171-expanded CD34(+) cord blood stem cells. *Blood*. 2017; 129(25):3344-3351.

110. Hernandez-Malmierca P, Vonficht D, Schnell A, et al. Antigen presentation safeguards the integrity of the hematopoietic stem cell pool. *Cell Stem Cell*. 2022;29(5):760-775.e10.

111. Kieusseian A, Brunet de la Grange P, Burlen-Defranoux O, Godin I, Cumano A. Immature hematopoietic stem cells undergo maturation in the fetal liver. *Development*. 2012;139(19):3521-3530.

112. Ema H, Nakauchi H. Expansion of hematopoietic stem cells in the developing liver of a mouse embryo. *Blood*. 2000;95(7): 2284-2288.

113. Ganuza M, Hall T, Myers J, et al. Murine foetal liver supports limited detectable expansion of life-long haematopoietic progenitors. *Nat Cell Biol*. 2022;24(10): 1475-1486.

114. de Bruijn MF, Speck NA, Peeters MC, Dzierzak E. Definitive hematopoietic stem cells first develop within the major arterial regions of the mouse embryo. *EMBO J*. 2000;19(11):2465-2474.

115. Gekas C, Dieterlen-Lievre F, Orkin SH, Mikkola HK. The placenta is a niche for hematopoietic stem cells. *Dev Cell*. 2005; 8(3):365-375.

116. Ottersbach K, Dzierzak E. The murine placenta contains hematopoietic stem cells within the vascular labyrinth region. *Dev Cell*. 2005;8(3):377-387.

117. Rhodes KE, Gekas C, Wang Y, et al. The emergence of hematopoietic stem cells is initiated in the placental vasculature in the absence of circulation. *Cell Stem Cell*. 2008; 2(3):252-263.

118. Li Z, Lan Y, He W, et al. Mouse embryonic head as a site for hematopoietic stem cell development. *Cell Stem Cell*. 2012;11(5): 663-675.

119. Nakano H, Liu X, Arshi A, et al. Haemogenic endocardium contributes to transient definitive haematopoiesis. *Nat Commun*. 2013;4:1564.

120. Bárcena A, Muench MO, Kapidzic M, Fisher SJ. A new role for the human placenta as a hematopoietic site throughout gestation. *Reprod Sci*. 2009;16(2):178-187.

121. Robin C, Bollerot K, Mendes S, et al. Human placenta is a potent hematopoietic niche containing hematopoietic stem and progenitor cells throughout development. *Cell Stem Cell*. 2009;5(4):385-395.

122. Charbord P, Tavian M, Humeau L, Péault B. Early ontogeny of the human marrow from long bones: an immunohistochemical study of hematopoiesis and its microenvironment. *Blood*. 1996;87(10):4109-4119.

123. Beaudin AE, Boyer SW, Perez-Cunningham J, et al. A transient developmental hematopoietic stem cell gives rise to innate-like B and T cells. *Cell Stem Cell*. 2016;19(6):768-783.

124. Lee LK, Ghorbanian Y, Wang W, et al. LYVE1 marks the divergence of yolk Sac definitive hemogenic endothelium from the primitive erythroid lineage. *Cell Rep*. 2016;17(9): 2286-2298.

125. Ludwig LS, Lareau CA, Ulirsch JC, et al. Lineage tracing in humans enabled by mitochondrial mutations and single-cell genomics. *Cell*. 2019;176(6):1325-1339.e22.

126. Azzoni E, Frontera V, Anselmi G, et al. The onset of circulation triggers a metabolic switch required for endothelial to hematopoietic transition. *Cell Rep*. 2021; 37(11):110103.

127. North TE, Goessling W, Peeters M, et al. Hematopoietic stem cell development is dependent on blood flow. *Cell*. 2009;137(4): 736-748.

128. Adamo L, Naveiras O, Wenzel PL, et al. Biomechanical forces promote embryonic haematopoiesis. *Nature*. 2009;459(7250): 1131-1135.

129. Bertrand JY, Chi NC, Santoso B, Teng S, Stainier DYR, Traver D. Haematopoietic stem cells derive directly from aortic endothelium during development. *Nature*. 2010;464(7285):108-111.

130. de Bruijn MFTR, Ma X, Robin C, Ottersbach K, Sanchez M-J, Dzierzak E. Hematopoietic stem cells localize to the endothelial cell layer in the midgestation mouse aorta. *Immunity*. 2002;16(5): 673-683.

131. Jaffredo T, Gautier R, Brajeul V, Dieterlen-Lièvre F. Tracing the progeny of the aortic hemangioblast in the avian embryo. *Dev Biol*. 2000;224(2):204-214.

132. Kissa K, Herbomel P. Blood stem cells emerge from aortic endothelium by a novel

type of cell transition. *Nature*. 2010; 464(7285):112-115.

133. Zovein AC, Hofmann JJ, Lynch M, et al. Fate tracing reveals the endothelial origin of hematopoietic stem cells. *Cell Stem Cell*. 2008;3(6):625-636.

134. Crosse EI, Gordon-Keylock S, Rybtsov S, et al. Multi-layered spatial transcriptomics identify secretory factors promoting human hematopoietic stem cell development. *Cell Stem Cell*. 2020;27(5): 822-839.e8.

135. Slukvin II. Generating human hematopoietic stem cells in vitro -exploring endothelial to hematopoietic transition as a portal for stemness acquisition. *FEBS Lett*. 2016; 590(22):4126-4143.

136. Wahlster L, Daley GQ. Progress towards generation of human haematopoietic stem cells. *Nat Cell Biol*. 2016;18(11):1111-1117.

137. Atkins MH, Scarfo R, McGrath KE, et al. Modeling human yolk sac hematopoiesis with pluripotent stem cells. *J Exp Med*. 2022;219(3):e20211924.

138. Bruveris FF, Ng ES, Leitoguinho AR, et al. Human yolk sac-like haematopoiesis generates RUNX1-GFI1- and/or GFI 1B-dependent blood and SOX17-positive endothelium. *Development*. 2020;147(20): dev193037.

139. Sturgeon CM, Ditadi A, Awong G, Kennedy M, Keller G. Wnt signaling controls the specification of definitive and primitive hematopoiesis from human pluripotent stem cells. *Nat Biotechnol*. 2014; 32(6):554-561.

140. Chanda B, Ditadi A, Iscove NN, Keller G. Retinoic acid signaling is essential for embryonic hematopoietic stem cell development. *Cell*. 2013;155(1):215-227.

141. Luff SA, Creamer JP, Valsoni S, et al. Identification of a retinoic acid-dependent haemogenic endothelial progenitor from human pluripotent stem cells. *Nat Cell Biol*. 2022;24(5):616-624.

142. Uenishi GI, Jung HS, Kumar A, et al. NOTCH signaling specifies arterial-type definitive hemogenic endothelium from human pluripotent stem cells. *Nat Commun*. 2018; 9(1):1828.

143. Fidanza A, Stumpf PS, Ramachandran P, et al. Single-cell analyses and machine learning define hematopoietic progenitor and HSC-like cells derived from human PSCs. *Blood*. 2020;136(25): 2893-2904.

144. Michaels YS, Edgar JM, Major MC, et al. DLL4 and VCAM1 enhance the emergence of T cell–competent hematopoietic progenitors from human pluripotent stem cells. *Sci Adv*. 2022;8(34): eabn5522.

145. de Leeuw S, Tackenberg C. Alzheimer's in a dish–induced pluripotent stem cell-based disease modeling. *Transl Neurodegener*. 2019;8:21.

146. Hicks MR, Pyle AD. The emergence of the stem cell niche. *Trends Cell Biol*. 2023;33(2): 112-123.

147. Fares I, Calvanese V, Mikkola HKA. Decoding human hematopoietic stem cell self-renewal. *Curr Stem Cell Rep*. 2022;8(2): 93-106.

148. Fares I, Chagraoui J, Gareau Y, et al. Pyrimidoindole derivatives are agonists of human hematopoietic stem cell self-renewal. *Science*. 2014;345(6203):1509-1512.

149. Boitano AE, Wang J, Romeo R, et al. Aryl hydrocarbon receptor antagonists promote the expansion of human hematopoietic stem cells. *Science*. 2010;329(5997): 1345-1348.

150. Sakurai M, Ishitsuka K, Ito R, et al. Chemically defined cytokine-free expansion of human haematopoietic stem cells. *Nature*. 2023;615(7950):127-133.

151. Butler JM, Gars EJ, James DJ, Nolan DJ, Scandura JM, Rafii S. Development of a vascular niche platform for expansion of repopulating human cord blood stem and progenitor cells. *Blood*. 2012;120(6): 1344-1347.

152. Bai T, Li J, Sinclair A, et al. Expansion of primitive human hematopoietic stem cells by culture in a zwitterionic hydrogel. *Nat Med*. 2019;25(10):1566-1575.

153. Zhang X, Cao D, Xu L, et al. Harnessing matrix stiffness to engineer a bone marrow niche for hematopoietic stem cell rejuvenation. *Cell Stem Cell*. 2023;30(4):378-395.e8.