REVIEW

Capturing embryonic hematopoiesis in temporal and spatial dimensions

Bart Weijts[a], and Catherine Robin[a,b]*

[a]Hubrecht Institute-KNAW and University Medical Center Utrecht, Utrecht, The Netherlands; [b]Regenerative Medicine Center, University Medical Center Utrecht, Utrecht, The Netherlands

Hematopoietic stem cells (HSCs) possess the ability to sustain the continuous production of all blood cell types throughout an organism's lifespan. Although primarily located in the bone marrow of adults, HSCs originate during embryonic development. Visualization of the birth of HSCs, their developmental trajectory, and the specific interactions with their successive niches have significantly contributed to our understanding of the biology and mechanics governing HSC formation and expansion. Intravital techniques applied to live embryos or non-fixed samples have remarkably provided invaluable insights into the cellular and anatomical origins of HSCs. These imaging technologies have also shed light on the dynamic interactions between HSCs and neighboring cell types within the surrounding microenvironment or niche, such as endothelial cells or macrophages. This review delves into the advancements made in understanding the origin, production, and cellular interactions of HSCs, particularly during the embryonic development of mice and zebrafish, focusing on studies employing (live) imaging analysis.

HIGHLIGHTS

- Imaging tools provide information with spatial and temporal dimension.
- Imaging provided evidence of the endothelial-to-hematopoietic (stem) cell transition in vertebrate embryo aortas.
- Heterogeneity of intra-aortic hematopoietic cluster cells is revealed by surface markers and transgenic reporter lines.

The adage "Seeing is believing" has significantly influenced scientific research, guiding the formulation and validation of hypotheses. The discovery of hematopoietic stem cells (HSCs), the cells at the foundation of the entire blood system, coincided with the evolution of microscopy during the early 20th century [1]. Integration of sophisticated microscopy techniques, fluorescent labeling methods, and advanced image analysis tools has enabled direct visualization of individual blood cells and tissue structures, such as bone marrow (BM) or blood vessels, thereby revolutionizing our comprehension of developmental and adult hematopoiesis. Continuous improvements in microscopy technologies as well as processing tools and software have increased imaging depth and resolution in complex tissue structures and embryos, facilitating optical sectioning, three-dimensional (3D) reconstructions, and prolonged imaging durations while minimizing phototoxicity and photobleaching [2,3]. Techniques like confocal, multiphoton, light sheet fluorescence, and intravital microscopy offer high spatiotemporal resolution imaging of large, living embryos or unfixed tissues in real-time, although each technique has its pros and cons. Fluorescent markers, including fluorescently tagged antibodies or transgenic reporter lines, aid in labeling specific hematopoietic cell populations for visualization and tracking. Imaging analysis software has become more user-friendly with point-and-click programs that allow processing and analysis of large datasets, with advanced options for quantification, tracking, and visualization of blood cell populations in time and space. Collectively, these technological advancements have opened new avenues for observing the dynamics of hematopoietic stem and progenitor cells (HSPCs) within their physiological environments in tissues or embryos, including cell-cell interactions, behaviors, and migration/homing. This review presents the latest findings on HSPC production, especially during mouse and zebrafish ontogeny, with a particular focus on how imaging approaches have contributed to establishing the endothelial origin of HSPCs in the embryonic dorsal aorta (DA) through an endothelial to hematopoietic transition (EHT), as well as the role of specialized niche cells in HSPC detachment after EHT, migration/homing, and quality control.

THE QUEST FOR THE SPATIAL AND TEMPORAL ORIGIN OF HSCs

The exploration of the spatial and temporal origins of HSCs began with the observation of the first blood cells, the primitive erythrocytes, in the extra-embryonic yolk sac (YS). Until the 1970s, this observation fueled the hypothesis that the YS served as the primary source of all blood cell production, including HSCs [4,5]. This hypothesis was

Address correspondence to Catherine Robin, Hubrecht Institute-KNAW and University Medical Center Utrecht, Uppsalalaan 8, 3584 CT, Utrecht, The Netherlands; E-mail: c.robin@hubrecht.eu

0301-472X/

https://doi.org/10.1016/j.exphem.2024.104257

further supported by a series of experiments using avian parabioses and *in vitro* assays to test the hematopoietic potential of mouse intra- and extra-embryonic tissues [6–12]. Sophisticated homotypic grafting experiments challenged the dogma of extra-embryonic origin of HSCs by tracing hematopoietic cells during ontogeny using avian YS chimeras [13–17]. Chicken/quail chimeras were created by grafting a whole embryo from one species onto the YS of another species at a comparable developmental age. Grafting was performed before the onset of circulation to prevent "contamination" by cells derived from one anatomical site, i.e., intra- or extra-embryonic, and migrating to another site. Relying on microscopy to distinguish chicken and quail cells through variances in the nucleoli (unique and compact for the quail, scattered and multiple for the chicken) and later on species-specific antibodies, it was shown that adult blood derived from an intra-embryonic source, while YS either did not contribute at all or only temporarily to cells that did not persist in adults [10,11,13,18,19].

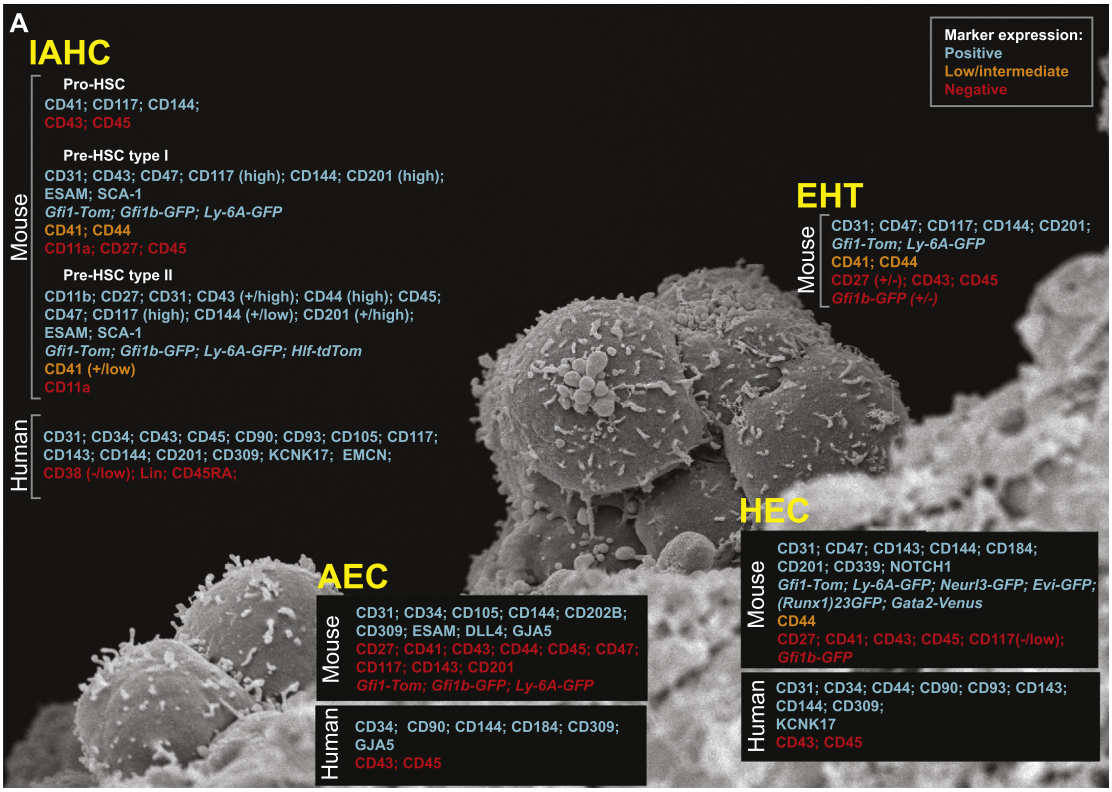
Although these pioneering avian studies pinpointed the region of the DA as the prospective source of HSPCs [20,21], confirmation of *bona fide* HSC presence in the DA of chicken embryos came more than 40 years later through an *in ovo* transplantation approach [22]. Transplanting green fluorescent protein-positive (GFP^+) chicken intra- or extra-embryonic tissues onto the chorioallantoic membrane of wild-type chicken embryos showed that only the embryonic tissue comprising the DA enabled lymphoid-myeloid multilineage reconstitution in recipients four months post-grafting, while grafting of YS, allantois, or head did not. This further established the critical role of the DA in housing the first HSCs produced during embryonic development. Grafting experiments in amphibians (Xenopus) confirmed that the dorsal lateral plate mesoderm (orthologous region of the aortic region) predominantly contributed to adult blood production, while the ventral blood island region (orthologous region of the YS) only contributed to embryonic blood production [23–25]. Evidence for the early developmental divergence of definitive and primitive blood was demonstrated by performing lineage tracing experiments in 32-cell stage Xenopus embryos [26,27].

From the above-mentioned avian and amphibian studies, the intra-embryonic DA was identified as the primary site of HSC production [28]. To test its validity in mammals, including humans, alternative strategies had to be developed due to the obvious impracticability of embryonic grafting strategies in mammals. Therefore, a series of *in vitro* and *in vivo* (e.g., short-term transplantation [spleen colony-forming unit {CFU-S} assay]) experiments confirmed that the para-aortic splanchnopleure, which sequentially develops into the aorta-gonad-mesonephros (AGM) region, generates definitive multipotent HSPCs in mouse and human embryos [29–34]. To date, injecting donor cells into irradiated adult recipients is considered the gold standard assay to determine whether cells have multilineage and self-renewal capacity and, therefore, are HSCs. Multipotency is analyzed in primary recipients in the long-term (after at least 4 months post-transplantation). The self-renewal capacity is evaluated through serial transplantations, where cells from the BM of primary reconstituted recipients are transplanted into secondary recipients and analyzed for multipotent progeny of donor origin. Using this *in vivo* transplantation assay, HSCs were detected in various sites starting at embryonic day (E) 10.5 of mouse development, i.e., the DA of the AGM region, the vitelline artery (VA), the umbilical artery (UA), and the head [35–39]. From E11 onward, HSCs are also found in the YS, placenta, and fetal liver (FL) [40–43]. At mid-gestation, the placenta and FL become important HSC reservoirs where HSCs mature and expand into a finite pool of HSCs with multilineage and self-renewal capacities before colonizing the BM starting at E17 [44]. In human embryos, HSCs with self-renewal and therefore regenerative capacity are also first detected in the AGM region, between 27 and 40 days of human embryonic development [45–48]. Yet, questions remained regarding the *de novo* generation of HSCs in specific anatomical sites, such as placenta and YS, and the identity of their direct precursors, prompting further investigation into alternative hypotheses.

HEMATOPOIETIC CLUSTERS CONTAINING THE FIRST HSCs LOCATE IN THE MAIN ARTERIES DURING A RESTRICTED DEVELOPMENTAL TIME WINDOW

Although the experimental confirmation of HSC activity in the main arteries of vertebrate embryos is relatively recent, the recognition of hematopoietic cells in that location dates back over a century. A meticulous examination of different fixed vertebrate embryos under the microscope revealed clusters of "hemoblast" cells, later referred to as intra-aortic hematopoietic clusters (IAHCs), in the DA of various embryo species [28,49–53]. Initially associated with a hematopoietic identity due to the high nuclear-to-cytoplasmic ratio of IAHC cells and an affinity for basophilic stains [21], this was later confirmed by immunostaining performed on fixed embryo sections or non-fixed thick slices, whole-mount embryos, or flow cytometry analyses after tissue dissociation using various combinations of hematopoietic markers. IAHCs appeared closely associated with the wall of the DA, composed of a layer of endothelial cells (ECs), facing the luminal side. IAHC cells exhibit a morphologically round shape, with bean, round, and ring-like nuclear shapes, distinct from the underlying flat ECs [54]. Scanning electron microscopy on thick embryo slices revealed the heterogeneity in size and shape of IAHCs, ranging from single cells to clusters of up to 19 cells, exhibiting either spheroidal, mushroom-like, or stacked organization, with all cells harboring microvilli on their surface (Figure 1A) [55]. Further observation using electron (scanning) microscopy underlined the ultrastructural similarities and tight junctions between IAHCs and the underlying ECs [55–57]. In contrast to the idea that IAHCs are just a random accumulation of circulating cells originating from other intra- or extra-embryonic regions, these observations led to the concept that they are more likely derived from the underlining endothelium. This implies that some ECs possess a hemogenic potential and are thus termed hemogenic ECs (HECs) [58,59].

Precise quantification, cartography, and organization of IAHCs along the circumference and length of the main arteries were initially performed by immunostaining on serial embryo sections collected along the anterior to posterior axis of the embryo and later by confocal imaging of whole-mount immunostained embryos of chicken [60,61], mouse [62], and human [63,64] species. The ability to acquire optical sections of an intact embryo allows for the software-based 3D reconstruction of the embryo, which preserves cell organization along the entire vasculature without disrupting tissue integrity or dislodging cells. By employing this approach, it was shown that IAHCs are randomly dispersed on the ventral aortic endothelium in avian and human embryos [60,63], although in mouse embryos, IAHCs are also found on the dorsal side (roof) of the DA, although less numerous [65]. Mouse IAHCs appear around E9.5 in the VA and shortly after in both DA and UA [66], distributed around the entire circumference and length of the VA, while IAHCs in UA are



B

Markers/Cluster of Differentiation	Description	Gene symbol
CD11a	Integrin Alpha L (ITGAL), the alpha subunit of LFA-1	*Itgal*
CD11b	Integrin Alpha M (ITGAM); the alpha subunit of Mac-1 (Macrophage-1 antigen)	*Itgam*
CD27	TNFRSF7 (Tumor necrosis factor receptor superfamily member 7)	*Cd27*
CD31	PECAM-1 (Platelet And Endothelial Cell Adhesion Molecule 1)	*Pecam-1*
CD34	Sialomucin/Mucosialin (hematopoietic progenitor and stem cell antigen)	*Cd34*
CD38	ADPRC 1 (ADP-Ribosyl Cyclase 1)	*Cd38*
CD41	Integrin subunit alpha IIb (Glycoprotein IIb)	*Itga2b*
CD43	Sialophorin (Leukosialin)	*Spn*
CD44	Extracellular matrix receptor III	*Cd44*
CD45	Protein Tyrosine Phosphatase Receptor Type C (LCA, leucocyte common antigen)	*Ptprc*
CD45RA	B220	*Ptprc*
CD47	Integrin Associated Protein (IAP)	*Cd47*
CD90	THY-1 (Cell surface antigen)	*Thy1*
CD93	AA4.1 antigen, C1QR1 (Complement component C1q receptor)	*Cd93*
CD105	Endoglin	*Eng*
CD117	c-Kit (KIT Proto-oncogene, Receptor tyrosine kinase, SCFR)	*Kit*
CD143	ACE (Angiotensin I converting enzyme)	*Ace*
CD144	VE-Cadherin (Vascular Endothelial Cadherin)	*Cdh5*
CD184	CXCR4 (CXC motif chemokine receptor type 4, SDF-1 receptor)	*Cxcr4*
CD201	Endothelial protein C receptor (EPCR, APC receptor)	*Procr*
CD202B	Angiopoietin-1 receptor (TIE2)	*Tek*
CD309	Kinase insert domain receptor (VEGFR2, FLK-1)	*Kdr*
CD339	Jagged Canonical Notch Ligand 1	*Jag1*
DLL4	Delta Like Canonical Notch Ligand 4	*Dll4*
EMCN	Endomucin	*Emcn*
ESAM	Endothelial Cell Adhesion Molecule	*Esam*
GJA5	Gap Junction Protein Alpha 5, Connexin 40	*Gja5*
KCNK17	Potassium Two Pore Domain Channel Subfamily K Member 17	*Kcnk17*
NOTCH1	Notch Receptor 1	*Notch1*
Evi1	MDS1 And EVI1 Complex Locus	*Mecom*
Gata2	GATA Binding Protein 2	*Gata2*
Gfi1	Growth Factor Independent 1 Transcriptional Repressor	*Gfi1*
Gfi1b	Growth Factor Independent 1B Transcriptional Repressor	*Gfi1b*
Ly-6A	SCA-1 (Stem cells antigen 1)	*Ly-6a*
Neurl3	Neuralized E3 Ubiquitin Protein Ligase 3	*Neurl3*
(Runx1) +23	*Runx1* +23 enhancer	*Runx1*

Figure 1 Commonly used surface markers and fluorescent transgenic lines to phenotypically characterize the endothelial and hematopoietic populations in the dorsal aorta of mouse and human embryos. **(A)** Scanning electron microscopy image of an E10-thick embryo slice, showing a close-up view of intra-aortic hematopoietic clusters (IAHCs) visible inside the aorta, in close contact with the endothelial wall. Markers expressed (positive, blue; low/intermediate, orange) and not expressed (negative, red) are indicated for aortic endothelial cells (AECs), hemogenic endothelial cells (HECs), cells undergoing endothelial to hematopoietic transition (EHT), and IAHC cells. IAHC cells include pro-hematopoietic stem cells (pro-HSCs) and precursors of HSCs type I and II (pre-HSCs). **(B)** Table listing all markers and transgenic lines referred to in the top panel, including their description and gene symbols.

predominantly located dorsally [49,54,62]. IAHCs in the DA are more concentrated around the central embryonic region [55,67,68], and are phenotypically similar but smaller than the ones in the UA and VA (e.g., up to 76 cells/cluster in the VA at E10.5) [62]. In chicken embryos, IAHCs are only present in the anterior region of the DA, restricted by the aortic arches and the DA-VA connection, but completely absent below this connection or in the VA [61]. In human embryos, IAHCs appear along the ventral endothelium of the VA around 30–36 days of development (Carnegie stage [CS]13–15), being more numerous at the DA-VA connection [63]. In the DA, IAHCs are also found along the ventral endothelium, more concentrated in the pre-umbilical section, emerging around 27 days (CS12) and persisting until 39–42 days (CS17) [69].

The connection between IAHCs and HSCs was established due to their shared expression of markers (e.g., c-Kit [62]) and their absence in *Runx1* knock-out embryos [70]. Although ventral and dorsal IAHCs are transcriptionally very similar [71], HSC activity is restricted to the ventral IAHCs, whereas committed hematopoietic progenitors (identified using *in vitro* clonogenic assay) are associated with both ventral and dorsal sides of the DA [65]. At E11.5, the DA harbors more functional HSCs, while small IAHCs of 1–2 cells are also predominant, preferentially expressing CD27 (TNFRSF7), which is known to be essential for all functional HSCs [72,73]. This observation fueled the idea that these small IAHCs are HSCs emerging directly from HECs. In support of a long-ignored correlation between IAHC size and HSC function, it was shown, either through Notch signaling perturbation [74] or knockout of *Svep1* [22] or *Pdgfrβ* [75], that large-size IAHCs correlate with decreased HSC activity. IAHC formation occurs during a restricted time window, with their number peaking at E10.5 with around 700 IAHC cells in the DA that disappear around E14.5 [62]. Human (35 days) and chicken (E3) DAs contain thousands of IAHC cells and their numbers also gradually decrease until 42 days (CS17) in human and E5.5 in chicken embryos [61,64]. Elegant quail-chicken transplantation experiments suggested that HECs originate from the splanchnopleural mesoderm, while the non-HECs originate from the paraxial (somitic) mesoderm [76]. Ultimately, the floor of the DA is replaced by the non-HECs, suggesting that HSCs were "lost" due to their emergence as IAHC cells. A similar process has been described for zebrafish (described below), although in mice it remains an open question.

PHENOTYPIC HETEROGENEITY OF IAHC CELLS

In vitro and *in vivo* functional assays as well as single-cell RNA (scRNA)-sequencing analysis of sorted endothelial and IAHC cells underlined the heterogeneity of IAHC cells, which in part seems to depend on IAHC size (as mentioned above) and the time point of development (described in details in reviews [69,73,77–83]. Successive precursors have been identified in IAHCs, where they mature via a multistep process, transitioning from pro-HSCs to pre-HSCs type I to pre-HSCs type II (between E9.5–E11.5, in the aorta) and finally to HSCs (mainly after migration in the FL) [84–86]. However, IAHCs also contain a mix of lymphoid, myeloid, and embryonic multipotent progenitors. To date, the exact cell composition of IAHCs is still under debate, depending on whether conclusions are based on transcriptomics or *in vitro*/*in vivo* functional data (reviewed in [81]). Moreover, it is still unclear whether all pre-HSCs will mature into functional HSCs.

Besides being functionally and transcriptionally heterogeneous (described in detail [79,81,82,87]), IAHC cells are also phenotypically heterogeneous. Conforming to their endothelial origin, multicolor stainings using various combinations of antibodies against surface markers have shown that IAHC cells co-express endothelial and hematopoietic markers, although cells are phenotypically heterogeneous within and between IAHCs (Figure 1A, B), e.g., [62,66,86,88–91]. Indeed, not all cells in IAHCs express the same markers or have the same levels of expression. For example, all IAHC cells express the hematopoietic marker c-Kit and endothelial markers CD31 and CD34, while other hematopoietic markers are restricted to a fraction of IAHC cells or are expressed with different levels of intensity at different time points of development, e.g., CD45, CD41, CD43, and endoglin [73,84,92–94]. Fluorescent reporter lines have also been useful to trace HEC and IAHC formation, including *Ly-6A-GFP* [66], *Gfi1-Tomato* and *Gfi1b-GFP* [95], *Neurl3-GFP* [96], *23-GFP* (*Runx1 +23* enhancer-reporter) [97], *Gata2-Venus* [98], and *Evi1-GFP* [99] (Figure 1A, B). Successive populations leading to the formation of HSCs have been phenotypically characterized, i.e., the aortic ECs (AECs), HECs, cells undergoing an EHT, and IAHC cells. A non-exhaustive summary of the recognized surface markers as well as markers used for fluorescent cell tracing is summarized in Figure 1A, B.

The functional assessment of cell proliferation in IAHCs was determined using fluorescent ubiquitination-based cell cycle indicator (FUCCI) reporter mice that enabled *in vivo* visualization of the cell-cycle status utilizing two anti-phase oscillating proteins that mark different phases of the cell cycle [100]. Cdt1-mKO2 is expressed during G0 and G1 phases (red fluorescence, slow cycling), and Geminin-mAG (Gem-mAG) is expressed during S/G2/M phases (green fluorescence, active cycling), while cells transiting from G1 to S phase appear yellow. Utilizing this FUCCI line, it was shown that pre-HSCs initially expand before they become more quiescent and start to acquire a definitive HSC state [101]. While slowly cycling cells are located at the base of IAHCs, actively proliferating cells are located at the more apical part of the cluster, where they highly express c-Kit and therefore are more receptive to c-Kit/SCF signaling for expansion [84,102].

Overall, IAHC cells exhibit phenotypic, functional, and transcriptional diversity within and between IAHCs, which can be in part attributed to the asynchronicity of the continuum of maturation. Therefore, isolating them to purity is difficult, and uncertainties remain on the exact expression level of some markers (e.g., negative, low, or intermediate), which can depend on the use of different fluorochromes conjugated to antibodies, flow cytometer sensitivity, and proper controls to determine the levels of expression.

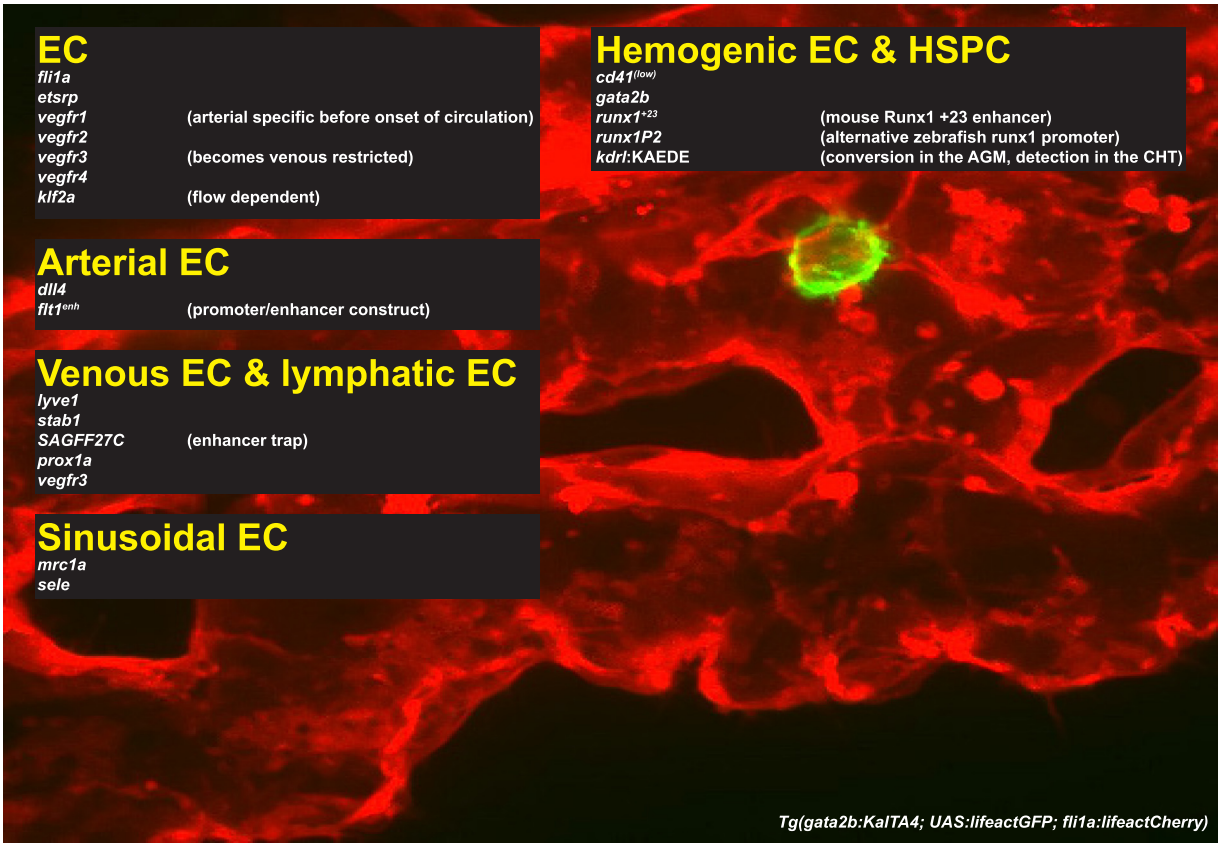
PROOF OF THE ENDOTHELIAL ORIGIN OF ALL HSCs

To establish the origins of the definitive hematopoietic system, various experimental approaches were pioneered, initially on avian embryos. Chicken embryos injected with a vital dye to label the entire vasculature showed for the first time that IAHC cells formed 1 day later were positive for both the pan-hematopoietic marker CD45 and the dye, proving their endothelial origin [60]. Due to rapid technological advances in microscopy, time-lapse imaging with short intervals and long durations allowed single-cell imaging and cell tracking. Such

methods enabled the visualization of mouse embryonic stem cells (ESCs) forming a hemogenic endothelium, from which nascent blood cells progressively bud off *in vitro* [103,104]. In mice, the definitive proof was established by using a VE-Cadherin Cre line to permanently label ECs. After induction, IAHCs in the DA and HSPC progeny in the FL and adult hematopoietic tissues (e.g., spleen, thymus, BM) were all labeled, demonstrating that all HSCs in adult mice derived from VE-Cadherin^{+} ECs [105]. In support of the HSC endothelial origin, endothelial-specific knockout of important hematopoietic transcription factors, i.e., RUNX1 or GATA2, resulted in a complete impairment of HSC production as well as IAHC formation [106,107].

VISUALIZING HSPC EMERGENCE FROM HEMOGENIC EC IN THE EMBRYONIC AORTA

Although the above-mentioned studies provided conclusive evidence for the endothelial origin of all HSCs, there was no absolute proof that HECs underwent a hematopoietic transition inside the



Markers	Description	Gene symbol
cd41	integrin, alpha 2b	*itga2b*
dll4	delta-like 4	*dll4*
etsrp	ETS1-related protein (etv2)	*etsrp*
fli1a	Fli-1 proto-oncogene, ETS transcription factor	*fli1*
flt1^{enh}	fms related receptor tyrosine kinase 1 (vegfr1)	*flt1*^{enh}
gata2b	GATA binding protein 2b	*gata2b*
kdrl	kinase insert domain receptor like	*kdrl*
klf2a	Krüppel like factor 2a	*klf2a*
lyve1	lymphatic vessel endothelial hyaluronic receptor 1	*lyve1*
mrc1a	mannose receptor, C type 1a	*mrc1a*
prox1a	prospero homeobox 1a	*prox1a*
runx1^{+23}	RUNX family transcription factor 1 (aml1), mouse Runx1 +23 enhancer	*runx1*
runx1P2	RUNX family transcription factor 1 (aml1), alternative zebrafish runx1 promoter	*runx1*
sele	selectin E	*sele*
stab1	stabilin 1	*stab1*
vegfr1	fms related receptor tyrosine kinase 1 (flt1)	*flt1*
vegfr2	kinase insert domain receptor (kdrb)	*kdr*
vegfr3	fms related receptor tyrosine kinase 4	*flt4*
vegfr4	kinase insert domain receptor like (flk1, kdra)	*kdrl*

Figure 2 Commonly used transgenic zebrafish lines to mark hematopoietic stem and progenitor cells (HSPCs) and different subsets of endothelial cells during zebrafish embryonic development. The background image shows a HSPC (green) in the caudal hematopoietic tissue region (red) of a *Tg(gata2b:KalTA4; UAS:lifeactGFP; fli1a:lifeactCherry)* zebrafish embryo 3 days post fertilization.

embryonic DA. Therefore, efforts have been made to image such a transition in real-time in the DA of embryos. In the past decades, zebrafish has gained significant traction as a model to study developmental hematopoiesis, as its *ex utero* development, the optical transparency of the embryo, and the availability to differentially mark HSPCs and the vasculature have enabled the visualization of cellular behaviors with a high spatiotemporal resolution (Figure 2). Time-lapse live confocal imaging of zebrafish embryos, in which GFP specifically marked the ECs, allowed to witness for the first time that flat aortic HECs located in the floor of the DA transitioned into round blood cells, a process referred to as endothelial-to-hematopoietic transition, or EHT [108]. Since then, the zebrafish model has been instrumental in further elucidating and dissecting the process of EHT. Direct imaging revealed that EHT is polarized not only in the dorso-ventral but also in the rostrocaudal versus mediolateral direction. Similar to mammals, and as reported with ESCs *in vitro* [104], HECs fail to complete EHT and undergo apoptosis in *runx1*-deficient embryos [108]. Unlike mammals, EHT does not result in HSPCs egressing into the aortic lumen to form IAHCs, but they rather emerge as single cells and transit into the mesenchyme of the sub-aortic space that separates the DA from the posterior cardinal vein (PCV) [108–110]. Analyses of the EHT process revealed that this transition is characterized by drastic morphological shape changes that start with a strong bending of the basal side of the HEC toward the sub-aortic space, creating a cup-shaped appearance. Even further bending of the cell allows the two neighboring ECs (left and right on the mediolateral axis) to connect with each other and establish tight junctions to maintain the vascular integrity of the DA when the HEC fully emerges from the vessel [108,111,112]. These alterations in cell shape are dependent on the contraction of an anisotropic circumferential actomyosin belt that facilitates the extruding of HECs.

To better understand the endothelial landscape of the DA and to map these morphologic changes, a software tool was developed, allowing the projection of lateral z-slices acquired in the anteroposterior orientation on a two-dimensional (2D) grid [111]. These 2D-maps revealed two main populations of ECs in the DA that were of similar length (in the anteroposterior direction) but significantly differed in width, with ECs that display a narrower appearance along the anteroposterior axis being hemogenic. The idea that ECs within the DA have different potentials was further supported by the fact that a portion of aortic ECs originate from a bipotential precursor in the somites that have no hemogenic potential [113,114]. HECs in the DA are thus seemingly solely derived from the splanchnic mesoderm located in the lateral plate mesoderm, while the supporting non-HECs originate from the dermomyotome located in the paraxial mesoderm. However, lineage tracing of a complete somite using photoconversion did show that some cells contributed to blood formation [115].

The emergence of HSPCs starts shortly after the onset of circulation, around 30 hours post fertilization (hpf), peaks approximately at 50 hpf, and continues until 70 hpf. Initiated by blood circulation, the DA undergoes drastic morphologic changes that are most likely required for EHT and include a ventralized EC redistribution, an initial rapid increase in DA diameter, and an adaptation of an irregular shape with regular intervals of thicker and thinner regions, referred to as corrugation [116]. The more than doubling diameter of the DA is not due to EC expansion but rather to cell shape changes [117]. Ultimately, the DA will return to its original diameter, and this is attributed to the loss of HECs that emerge as HSPCs [111,117].

At the same time that EHT was visualized in zebrafish embryos, pioneering work was done to capture the birth of HSCs from the DA in mouse embryos, which required overcoming the problem of accessing the DA, which is deeply located in opaque embryos that develop within the uterus. An elegant solution to this problem was to image thick transversal slices from non-fixed E10.5 embryos. To visualize the entire endothelium, the embryos were injected with fluorescent antibodies (i.e., against CD31) before imaging [118,119]. Using *CD41-YFP* and *Ly-6A(Sca-1)-GFP* reporter lines that mark nascent HSCs, *CD41-YFP*$^{+}$ and *Ly-6A-GFP*$^{+}$ cells were observed budding directly from the endothelium toward the lumen side, while they retained the CD31 staining, thus proving their endothelial origin. Additional staining of the slides at the end of imaging and re-imaging of the same location revealed that these *CD41-YFP*$^{+}$ and *Ly-6A-GFP*$^{+}$ cells also co-express c-Kit, validating the HSC phenotypic signature. The estimated frequency of emergence events was extremely low (<2 per embryo), which might be due to the absence of blood flow or shear stress known to be essential for HSC production *in vivo* [120–123]. In addition, no cells emerged from the DA of embryos younger than E10.5 (<33 somite pairs) or the DA of *Runx1* knockout embryos, further supporting the validity of the experimental and imaging techniques. This *ex vivo* imaging approach of non-fixed embryo thick slices has since been instrumental in new findings in the formation of IAHCs in the mouse DA. For example, imaging of *VE-Cadherin*-CreER: Confetti$^{fl/fl}$ embryo slices shed light on the clonal composition of IAHCs, showing that after EHT, proliferative and monoclonal IAHCs are formed, which then progressively become more polyclonal via the recruitment of neighboring HECs [74], as also shown by others [124]. The size of IAHCs is in part determined by the number of HECs recruited into the IAHCs, a process depending on NOTCH signaling [74]. Another example was the observation that *Gata2* expression oscillates in cells undergoing EHT in embryo slices [125]. These results led to the hypothesis that the acquisition of a hematopoietic fate occurs stepwise and that a signal window is opened in each step in which *Gata2* is essential to maintaining the HSC fate, but other fate decisions can take place, such as the formation of lineage-committed progenitors [82,87]. Overall, the *ex vivo* imaging of embryo slices has further supported the hypothesis that HSPCs are generated from HECs located in the ventral part of the DA. However, questions remain regarding the process underlying EHT and HSC generation in the mouse DA and whether (some) observations made in zebrafish embryos can be translated to mice or humans, knowing that HSPCs emerge in the opposite direction and without the formation of IAHCs in zebrafish. Of note, in chicken embryos, although most hematopoietic cells delaminating from the aortic floor are released into the bloodstream, some go in the opposite direction and ingress into the dorsal mesentery [126]. Another intriguing question is whether extra-embryonic sites (e.g., YS or placenta) are also capable of *de novo* HSC production via an endothelial transition, similar to the DA in mammals.

IAHC-LIKE STRUCTURES IN ATYPICAL (NON-HEMATOPOIETIC) ANATOMICAL SITES

Immunostaining on whole-mount/cryosections and confocal microscopy of extra- and intra-embryonic tissues from E7.5 onward showed the presence of IAHC-like structures in other hematopoietic tissues, besides arteries, as well as in non-hematopoietic sites. Dispersed

hematopoietic clusters ($CD31^+RUNX1^+c\text{-}Kit^+$) are found in the proximal region of the vascular plexus in both the arterial and venous vessels of YS from E9.5 mouse embryos [54,127]. By E10.5, distinct clusters of hematopoietic cells are found in small-diameter arterial and venous vessels throughout the proximal and distal regions of the YS. IAHC-like structures have also been reported in the vasculature of mouse and human placenta [128–130]. Hematopoietic clusters are also present in the intersomitic vessels and the dorsal longitudinal anastomotic vessels of E10.5 mouse embryos [54]. Large IAHC-like structures are found in the ventricular cavity of the heart of E10.5 mouse embryos, as well as in the atrioventricular canal [54]. Some endocardial cells might have hemogenic potential [131,132], although this finding was not confirmed in a recent study [133]. HSPC generation via EHT was long thought to be restricted to the early stages of development. Using interspecific grafts, genetic tagging, and live imaging, HECs with a molecular signature similar to that of embryonic aortic HECs have been identified in the BM of late fetus/young adult chickens and mice, where they can generate *de novo* HSPCs via EHT [134].

The notion that *de novo* production of HSPCs can occur in atypical anatomic sites is interesting and could be the basis for our understanding of the microenvironment requirements for the generation of HSPCs, but additional experiments will be required to further support these claims. For example, despite the presence of HSCs in the head at E10.5, identified by performing long-term transplantations [36], a combination of whole-mount immunostaining and 3D confocal reconstruction techniques of the embryonic head revealed the absence of RUNX1 expression in the vasculature, supporting the absence of HECs, EHT events and hematopoietic cluster formation, overall suggesting that HSCs may not be *de novo* generated from the head endothelium [135,136]. Volumetric imaging of whole mouse embryos showed the presence of extravascular islands that most likely result from the extravascular budding of hematopoietic clusters in the VA and the UA that might contribute to either hematopoiesis [137] or the formation of new blood vessels [138]. However, other imaging analyses suggest that hematopoietic clusters remain intravascular during the period of vascular remodeling [139]. Overall, live imaging combined with a more sophisticated lineage-tracing experiment would be of great interest to confirm or refute some of the above-mentioned findings in several non-hematopoietic sites. Functional assays also need to be performed to determine and validate their hematopoietic potential.

THE INFLUENCE OF SHEAR STRESS IN HSPC EMERGENCE IN THE DA

As previously stated for mammals, blood flow-induced shear stress in the embryonic DA is an important regulator of HSC formation. New microscopic observations in wild-type zebrafish embryos, embryos with reduced or absent blood flow (silent-heart mutants), and those with disrupted actomyosin machinery revealed that mechanical forces not only serve as signals promoting and synchronizing EHT but also assist the process by activating shape instabilities directly [140]. In zebrafish embryos, without blood flow or perturbed flow, there is a significant reduction in EC redistribution toward the ventral side of the DA, EC shape changes, and HSPC emergence [117,121,141]. Hemodynamic forces/mechanical cues created by blood flow are important for proper vascular homeostasis, EC remodeling, and the formation of HSCs and thus require proper sensing and relaying of these forces into a cellular response. Primary cilia, hairy-like sensory organelles composed of microtubules, are versatile structures that, among others, can sense flow and flow direction through the deflection of the cilia and the activation of mechanotransduction signaling (reviewed in [142]). Some HECs ($runx1^+$) located in the ventral floor of the DA are ciliated, and disruption of *fsd1* (fibronectin type III and SPRY domain-containing 1), a gene coding for a centrosome-associated protein linked to a subset of microtubules and required for ciliogenesis, results in a reduction in $runx1^+$ cells in the floor of the DA and HSPC emergence [143]. However, fluorescence *in situ* hybridization (FISH) for *c-myb* and cilia showed that the $c\text{-}myb^+$ HECs in the AGM region were not ciliated [143], suggesting that some HECs have alternative methods for flow sensing. One such transducer of physical stimuli into HECs is the transcriptional coregulator Yes-activated protein (YAP), which shuttles from the cytoplasm into the nucleus in a blood flow-dependent manner to facilitate EHT through the regulation of *runx1* and *c-myb* [144]. Another flow-dependent pathway that has been shown to be involved in HSPC formation is the krüppel-like transcription factor 2a (Klf2a), which directly regulates nitric oxide (NO) synthase and leads to the production and secretion of NO by the ECs, which in turn regulates HSPC formation [121,145,146]. Notch signaling is yet another pathway that can be activated by blood flow and is critical for HEC specification and HSPC production (review [78]). Therefore, it is reasonable to assume that blood flow might be involved in the activation and/or maintenance of the Notch signal in the DA, including the HECs and HSPCs [147,148]. The initial wave of Notch activation in the nascent HSPC population occurs at an early stage of embryonic development, captured by imaging of the hemangioblasts migrating from the lateral side of the embryo toward the midline, where these cells closely migrate across the ventral face of the somite, expressing the Notch ligands Dlc and Dld [149]. Notch activation is then maintained via various routes, including the possibility of blood flow-induced Notch activation in HSPCs and neutrophil-dependent activation of Notch [150].

HSPC INTERACTIONS WITH ECs AND MACROPHAGES IN GENERATION AND EXPANSION NICHES

Imaging has played an important role in visualizing the cellular interactions of HSPCs in the DA, as well as in secondary hematopoietic sites where HSCs mature and expand. Immune cells play an important role in HSPC emergence, maintenance, and guidance, with a pronounced role for macrophages. At the time of HSPC emergence in zebrafish embryos, macrophages accumulate in the sub-aortic space, where they intensively interact with HSPCs and secrete matrix metalloproteinases that modify the extracellular matrix to facilitate EHT, their migration in the AGM stroma, and the intravasation of the PCV to enter the circulation [151,152]. Similarly, it was shown in mice that macrophages localized in the aorta expressed a pro-inflammatory signature and dynamically interacted with nascent and emerging intra-aortic hematopoietic cells [153]. In zebrafish, HSPCs migrate to the caudal hematopoietic tissue (CHT) after they enter the circulation. The CHT is the equivalent of the FL in mammals and is composed of a transient network of venous sinusoids in the caudal part of the tail, where HSPCs differentiate and expand before they migrate again to seed the definitive hematopoietic organs, the thymus

and kidney marrow (the equivalent to the BM in mammals) [154]. Upon their arrival in the CHT, HSPCs interact with the sinusoidal ECs and transmigrate across the vascular barrier, where they trigger the remodeling of their direct microenvironment to create a pocket, or niche, that mainly consists of sinusoidal ECs and fibroblastic reticular cells, a type of stromal cell [154,155]. A similar process also seems to occur in mouse FL, as shown by imaging liver slices *ex vivo* [155]. Niche factors involved in homing and/or HSPC expansion include *cxcl8, cxcl12, ccl25b, gpr182, kitlb, thrombopoietin, csf1a*, and *erythropoietin* [155–160]. Macrophages, or so-called usher cells, patrol the sinusoidal vessels for HSPCs, retain them upon contact, and facilitate their extravasation into the stroma [161]. In addition, macrophage-HSPC interaction is required to maintain a healthy population of HSPCs, as stressed stem cells are targeted and phagocytosed by the macrophages in the CHT, a process called grooming and dooming [162]. There is a possibility that these macrophages also play a role in the modulation of the niche itself through the secretion of *mmp9* and by modulating *cxcr1* expression in niche ECs [152,158].

To fully understand the precise cellular composition of the HSPC niche and to dissect the required signaling events to maintain and expand HSPCs will still take a tremendous amount of work, but it will be essential to improve current protocols to expand HSPCs *in vitro*. A comprehensive single-cell transcriptomic atlas of HSPC expansion in the zebrafish CHT was recently created, which can now be used as a resource for future functional studies [160]. The fact that an ectopic HSPC vascular niche can be recreated outside of the context of the CHT by reprogramming muscle, neurons, or skin cells by the overexpression of a 3-factor combination of transcription factors (Etv2, Sox7, and Nr2f2) suggests that under the right conditions, a *bona fide* niche can be induced in which HSPCs can be maintained and expanded [163]. Recently, researchers also developed workflows to integrate fluorescent live imaging and light sheet microscopy to generate a 3D visualization of the entire kidney marrow of zebrafish larvae and to visualize HSPC lodging [164]. In parallel, they also performed a series of sophisticated sectioning approaches on zebrafish to generate 3D datasets of about 3,000 tissue sections, each coupled to high-resolution imaging at the sub-cellular scale. Such analysis heavily based on imaging allowed to define different HSPC niches adjacent to the glomerulus in the anterior kidney region and the posterior vascular and perivascular regions. HSPCs are in close contact with the nervous system, which is involved in HSPC regulation, with ECs and stromal cells, as well as with other HSPCs, as is also the case in the CHT during embryonic development [155].

CONCLUDING REMARKS AND PERSPECTIVES

For decades, imaging has been the basis of our understanding of the production of hematopoietic cells *in vivo* or *in vitro* and has enabled significant discoveries through the continuous improvement of tools, optical components, and analysis techniques. However, many questions remain unanswered regarding the formation and regulation of IAHCs and HSPCs during embryonic development, primarily because the anatomical sites where these processes occur are not easily accessible, particularly in mammalian embryos, or difficult to reproduce *in vitro*. Discrepancies between findings often arise from variations in assays, investigative strategies, and/or data interpretation, i.e., comparing, for example, findings from lineage-tracing experiments and transplantation assays. In part, some of the discrepancies and variations can be attributed to the lack of specific antibodies and the reliance on the use of reporter lines for the zebrafish. For example, the random integration of reporter cassettes via the tol2 transposon system can result in ectopic regulation of the promoter. Upstream activating sequence (UAS) systems are often prone to silencing, and due to the relatively long lifetime of fluorescent protein cells, can be mislabeled [165,166].

Various hypotheses revolve around the timing and location at which a cell acquires hemogenic potential, (pro/pre-)HSC properties, or a more committed fate (e.g., hematopoietic progenitors, mature cells). The intrinsic and extrinsic factors involved in these processes remain elusive. Additionally, the lack of specific or unique markers for HECs with different hematopoietic potentials poses a challenge, as does the need for reliable markers to distinguish and trace different stages of hematopoietic development. Despite most blood cells deriving from HECs, questions persist regarding the precise timing and frequency of EHT events and whether *de novo* EHT occurs in all sites where clusters and/or HSCs are detected, particularly in mammals. There is also the possibility of inducing the hemogenic capacity of other types of endothelium, such as lymphatic endothelium [167]. Despite recent progress, the journey to understand the regulation and cellular interactions occurring when EHT takes place and how the newly formed HSPCs migrate and home from the aorta to successive hematopoietic sites, such as the FL in mice or the CHT in zebrafish embryos, has just begun. Some reports based on lineage-tracing experiments suggest that hematopoietic progenitors, rather than HSCs, are the dominant contributor to both embryonic and young adult hematopoiesis under physiological conditions [168,169], raising the possibility that embryos/adults could survive with only multipotent progenitors. On the other hand, studies have shown that erythroid-myeloid progenitors (EMPs) produced in the YS are the origin of most tissue-resident macrophages in adults, emphasizing the importance of comprehensive studies on developmental hematopoiesis.

The ultimate objective of regenerative medicine is to generate *in vitro* HSCs with full transplantation capacity, akin to those found *in vivo*. Indeed, multipotent progenitors do not engraft in the long-term and cannot replace defective HSCs or defective blood systems in patients with blood-related diseases upon transplantation. Real-time *in vivo* examination of the myriad intrinsic and extrinsic signals affecting HEC fate could elucidate their heterogeneity and capacity to produce specific hematopoietic cell types, in particular HSCs [82]. Regulatory mechanisms, including metabolic changes, NO signaling, (blood) flow-induced shear stress, and biomechanical forces, are increasingly recognized as key players in controlling stem cell fate. Understanding how these factors are dynamically orchestrated to control cell fate is crucial for the *in vitro* production of clinically transplantable HSCs or for developing treatment strategies for blood-related diseases. Stringent regulations surrounding the use of mammalian embryos, particularly humans, have prompted the development of pluripotent stem cell-derived models. Thus, in addition to advancing *in vivo* experimental models, efforts are being made to develop *in vitro* systems such as the BM [170], liver [171] or blood vessel organoids [172], whole embryo-like structures named gastruloids [173], and AGM-on-a-chip model [174], offering less complexity, greater accessibility (e.g., for imaging), and mimicking certain aspects of *in vivo* counterparts as an arterial (hemogenic) endothelial network or a supportive HSPC environment. Although these complex 3D models aim to replicate crucial aspects of genetic, biochemical, and

mechanical processes occurring during embryonic development, they are not exact replicas of natural embryos or hematopoietic organs. Still, they offer a valuable opportunity for scientists to study human development and observe and manipulate developmental phenomena that may be challenging to access otherwise. It is also crucial to recognize that these models can be used for intercellular interactions, early developmental decisions, and to probe crosstalk between healthy and unhealthy tissues. However, insights gained from *in vitro* studies must be thoroughly validated in real embryos before concluding and to ensure their reliability and relevance.

Future studies will require a combination of live imaging and lineage tracing using reliable (and ideally unique) markers to capture the fate transition process accurately during developmental and postnatal hematopoiesis. The quest to understand developmental hematopoiesis will continue through the utilization of mathematical and predictive models, along with innovative (quantitative) imaging techniques employing animal models such as zebrafish embryos. Innovations in imaging techniques aim to enhance access to larger, deeper samples while also striving for increased speed and sensitivity. Furthermore, improvements in sample and embryo preparation, including enhanced transparency (albeit only on fixed specimens) and the use of a broader spectrum and brighter fluorophores, will enhance our ability to discriminate, lineage trace, and manipulate different cells in one specimen. Integrating multimodal omics data will be crucial for determining cell fate decisions and generating hematopoietic organoids or gastruloids to recapitulate HSC induction and expansion under native conditions. Direct (quantitative) imaging is a powerful approach not only to obtain *in situ* information but also to provide guidance for predictive models and to design perturbation assays [175]. The ultimate aspiration is to capture multiple biological processes in action across multiple dimensions (space and time), within numerous samples or living embryos. Automated standardized processes and analysis tools, along with the integration of artificial intelligence, will facilitate efficient data translation and sharing. Moreover, the sheer volume of information generated (i.e., imaging, transcriptomics, proteomics, experimental, and bibliography) will necessitate the use of well-trained artificial intelligence to efficiently manage, process, and connect data, given that our brain capacities may be insufficient for such tasks!

Conflict of Interest Disclosure

The authors do not have any conflicts of interest to declare in relation to this work.

Acknowledgments

This work was supported by the KWF Dutch Cancer Society (13549), NWO Dutch Research Council (OCENW.M20.178), and Landsteiner Foundation for Blood Transplantation Research (LSBR 22860).

Author Contributions

BW and CR: conceptualization, investigation, writing, reviewing, and editing.

REFERENCES

1. Klaus A, Robin C. Embryonic hematopoiesis under microscopic observation. Dev Biol 2017;428:318–27.
2. Daetwyler S, Fiolka RP. Light-sheets and smart microscopy, an exciting future is dawning. Commun Biol 2023;6:502.
3. Balasubramanian H, Hobson CM, Chew TL, Aaron JS. Imagining the future of optical microscopy: everything, everywhere, all at once. Commun Biol 2023;6:1096.
4. Dantschakoff V. Uber das erste Auftreten der Blutelemente beim Hühnerembryo. Folia Haematol 1907;4:159–66.
5. Maximow A. Untersuchengenuber blut und bindgewebe: 1. Die fruhesten entwicklungs-stadian der Blut- und Bindgewebszellen beim saugetierembryo, bis sum anfang der blutbildung in der leber. Arch Mikr Anat Entwicklungsgesch 1909;73:444–561.
6. Moore MA, Owen JJ. Chromosome marker studies on the development of the haemopoietic system in the chick embryo. Nature 1965;208:956.. passim.
7. Moore MA, Metcalf D. Ontogeny of the haemopoietic system: yolk sac origin of in vivo and in vitro colony forming cells in the developing mouse embryo. Br J Haematol 1970;18:279–96.
8. Moore MA, Owen JJ. Chromosome marker studies in the irradiated chick embryo. Nature 1967;215:1081–2.
9. Moore MA, Owen JJ. Experimental studies on the development of the thymus. J Exp Med 1967;126:715–26.
10. Lassila O, Eskola J, Toivanen P, Martin C. Dieterlen-Lievre F. The origin of lymphoid stem cells studied in chick yold sac-embryo chimaeras. Nature 1978;272:353–4.
11. Lassila O, Martin C, Toivanen P, Dieterlen-Lievre F. Erythropoiesis and lymphopoiesis in the chick yolk-sac-embryo chimeras: contribution of yolk sac and intraembryonic stem cells. Blood 1982;59:377–81.
12. Weissman I, Papaioannou V, Gardner R. Fetal hematopoietic origins of the adult hematolymphoid system. In: Clarkson B, Mark P, Till J, eds. Differentiation of normal and neoplastic cells, New York: Cold Spring Harbor Laboratory Press; 1978:33–47.
13. Dieterlen-Lievre F. On the origin of haemopoietic stem cells in the avian embryo: an experimental approach. J Embryol Exp Morphol 1975;33:607–19.
14. Le Douarin NM, Dieterlen-Lievre F. How studies on the avian embryo have opened new avenues in the understanding of development: a view about the neural and hematopoietic systems. Dev Growth Differ 2013;55:1–14.
15. Le Douarin NM, Jotereau FV, Houssaint E, Belo M. Ontogeny of the avian thymus and bursa of Fabricius studied in interspecific chimeras. Ann Immunol (Paris) 1976;127:849–56.
16. Beaupain D, Martin C. Dieterlen-Lievre F. Are developmental hemoglobin changes related to the origin of stem cells and site of erythropoiesis? Blood 1979;53:212–25.
17. Martin C, Beaupain D. Dieterlen-Lievre F. Developmental relationships between vitelline and intra-embryonic haemopoiesis studied in avian 'yolk sac chimaeras'. Cell Differ 1978;7:115–30.
18. Lassila O, Martin C, Dieterlen-Lievre F, Nurmi TE, Eskola J, Toivanen P. Is the yolk sac the primary origin of lymphoid stem cells? Transplant Proc 1979;11:1085–8.
19. Martin C, Lassila O, Nurmi T, Eskola J, Dieterlen-Lievre F, Toivanen P. Intraembryonic origin of lymphoid stem cells in the chicken: studies with sex chromosome and IgG allotype markers in histocompatible yolk sac-embryo chimaeras. Scand J Immunol 1979;10:333–8.
20. Cormier F, Dieterlen-Lievre F. The wall of the chick embryo aorta harbours M-CFC, G-CFC, GM-CFC and BFU-E. Development 1988;102:279–85.
21. Dieterlen-Lievre F, Martin C. Diffuse intraembryonic hemopoiesis in normal and chimeric avian development. Dev Biol 1981;88:180–91.
22. Yvernogeau L, Klaus A, Maas J, et al. Multispecies RNA tomography reveals regulators of hematopoietic stem cell birth in the embryonic aorta. Blood 2020;136:831–44.

23. Kau CL, Turpen JB. Dual contribution of embryonic ventral blood island and dorsal lateral plate mesoderm during ontogeny of hemopoietic cells in Xenopus laevis. J Immunol 1983;131:2262–6.
24. Maeno M, Tochinai S, Katagiri C. Differential participation of ventral and dorsolateral mesoderms in the hemopoiesis of Xenopus, as revealed in diploid-triploid or interspecific chimeras. Dev Biol 1985;110:503–8.
25. Turpen JB, Knudson CM, Hoefen PS. Embryonic origin of hematopoietic precursor cells from the region of presumptive mesonephros in Rana pipiens. Anat Rec 1981;199:261A.
26. Ciau-Uitz A, Walmsley M, Patient R. Distinct origins of adult and embryonic blood in Xenopus. Cell 2000;102:787–96.
27. Turpen JB, Knudson CM, Hoefen PS. The early ontogeny of hematopoietic cells studied by grafting cytogenetically labeled tissue anlagen: localization of a prospective stem cell compartment. Dev Biol 1981;85:99–112.
28. Dieterlen-Lievre F, Pouget C, Bollerot K, Jaffredo T. Are intra-aortic hemopoietic cells derived from endothelial cells during ontogeny? Trends Cardiovasc Med 2006;16:128–39.
29. Medvinsky AL, Samoylina NL, Muller AM, Dzierzak EA. An early pre-liver intraembryonic source of CFU-S in the developing mouse. Nature 1993;364:64–7.
30. Godin IE, Garcia-Porrero JA, Coutinho A, Dieterlen-Lievre F, Marcos MA. Para-aortic splanchnopleura from early mouse embryos contains B1a cell progenitors. Nature 1993;364:67–70.
31. Godin I, Dieterlen-Lievre F, Cumano A. Emergence of multipotent hemopoietic cells in the yolk sac and paraaortic splanchnopleura in mouse embryos, beginning at 8.5 days postcoitus. Proc Natl Acad Sci U S A 1995;92:773–7.
32. Cumano A, Dieterlen-Lievre F, Godin I. Lymphoid potential, probed before circulation in mouse, is restricted to caudal intraembryonic splanchnopleura. Cell 1996;86:907–16.
33. Cumano A, Ferraz JC, Klaine M, Di Santo JP, Godin I. Intraembryonic, but not yolk sac hematopoietic precursors, isolated before circulation, provide long-term multilineage reconstitution. Immunity 2001;15:477–85.
34. Tavian M, Robin C, Coulombel L, Peault B. The human embryo, but not its yolk sac, generates lympho-myeloid stem cells: mapping multipotent hematopoietic cell fate in intraembryonic mesoderm. Immunity 2001;15:487–95.
35. 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;19:2465–74.
36. Li Z, Lan Y, He W, et al. Mouse embryonic head as a site for hematopoietic stem cell development. Cell Stem Cell 2012;11:663–75.
37. Medvinsky A, Dzierzak E. Definitive hematopoiesis is autonomously initiated by the AGM region. Cell 1996;86:897–906.
38. Muller AM, Medvinsky A, Strouboulis J, Grosveld F, Dzierzak E. Development of hematopoietic stem cell activity in the mouse embryo. Immunity 1994;1:291–301.
39. Gordon-Keylock S, Sobiesiak M, Rybtsov S, Moore K, Medvinsky A. Mouse extraembryonic arterial vessels harbor precursors capable of maturing into definitive HSCs. Blood 2013;122:2338–45.
40. Gekas C, Dieterlen-Lievre F, Orkin SH, Mikkola HK. The placenta is a niche for hematopoietic stem cells. Dev Cell 2005;8:365–75.
41. Kumaravelu P, Hook L, Morrison AM, et al. Quantitative developmental anatomy of definitive haematopoietic stem cells/long-term repopulating units (HSC/RUs): role of the aorta-gonad-mesonephros (AGM) region and the yolk sac in colonisation of the mouse embryonic liver. Development 2002;129:4891–9.
42. Ottersbach K, Dzierzak E. The murine placenta contains hematopoietic stem cells within the vascular labyrinth region. Dev Cell 2005;8:377–87.
43. Alvarez-Silva M, Belo-Diabangouaya P, Salaun J. Dieterlen-Lievre F. Mouse placenta is a major hematopoietic organ. Development 2003;130:5437–44.
44. Christensen JL, Wright DE, Wagers AJ. Weissman IL. Circulation and chemotaxis of fetal hematopoietic stem cells. PLoS Biol 2004;2:E75.
45. Sinka L, Biasch K, Khazaal I, Peault B, Tavian M. Angiotensin-converting enzyme (CD143) specifies emerging lympho-hematopoietic progenitors in the human embryo. Blood 2012;119:3712–23.
46. Ivanovs A, Rybtsov S, Welch L, Anderson RA, Turner ML, Medvinsky A. Highly potent human hematopoietic stem cells first emerge in the intra-embryonic aorta-gonad-mesonephros region. J Exp Med 2011;208:2417–27.
47. Catlin SN, Busque L, Gale RE, Guttorp P, Abkowitz JL. The replication rate of human hematopoietic stem cells in vivo. Blood 2011;117:4460–6.
48. 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;15:811–6.
49. Garcia-Porrero JA, Godin IE, Dieterlen-Lievre F. Potential intraembryonic hemogenic sites at pre-liver stages in the mouse. Anat Embryol (Berl) 1995;192:425–35.
50. Jordan HE. Aortic cell clusters in vertebrate embryos. Proc Natl Acad Sci U S A 1917;3:149–56.
51. Dantschakoff W. Untersuchungen über die Entwicklung von Blut und Bindegewebe bei Vö geln. Das lockere Bindegewebe des Hühnchens im Fetalen Leben. Arch f mikr Anat. 1909;73:117–81.
52. Jordan HE. A study of 7mm human embryo; with special reference to its peculiar spirally twisted form and its large aortic cell clusters. Anat Rec 1918;14:479–92.
53. Emmel V. The cell clusters in the dorsal aorta of mammalian embryos. Am J Anat 1916;19:401–16.
54. Yzaguirre AD, Speck NA. Insights into blood cell formation from hemogenic endothelium in lesser-known anatomic sites. Dev Dyn 2016;245:1011–28.
55. Boisset JC, Clapes T, Klaus A, et al. Progressive maturation toward hematopoietic stem cells in the mouse embryo aorta. Blood 2015;125:465–9.
56. Smith RA, Glomski CA. Hemogenic endothelium" of the embryonic aorta: Does it exist? Dev Comp Immunol 1982;6:359–68.
57. Bos FL, Hawkins JS. Zovein AC. Single-cell resolution of morphological changes in hemogenic endothelium. Development 2015;142:2719–24.
58. Jordan HE. Evidence of hemogenic capacity of endothelium. Anat Rec 1916;10:417–20.
59. Wu Y, Hirschi KK. Regulation of hemogenic endothelial cell development and function. Annu Rev Physiol 2021;83:17–37.
60. Jaffredo T, Gautier R, Eichmann A. Dieterlen-Lievre F. Intraaortic hemopoietic cells are derived from endothelial cells during ontogeny. Development 1998;125:4575–83.
61. Yvernogeau L, Robin C. Restricted intra-embryonic origin of bona fide hematopoietic stem cells in the chicken. Development 2017;144:2352–63.
62. Yokomizo T, Dzierzak E. Three-dimensional cartography of hematopoietic clusters in the vasculature of whole mouse embryos. Development 2010;137:3651–61.
63. Tavian M, Coulombel L, Luton D, Clemente HS, Dieterlen-Lievre F, Peault B. Aorta-associated CD34+ hematopoietic cells in the early human embryo. Blood 1996;87:67–72.
64. Tavian M, Hallais MF, Peault B. Emergence of intraembryonic hematopoietic precursors in the pre-liver human embryo. Development 1999;126:793–803.
65. Taoudi S, Medvinsky A. Functional identification of the hematopoietic stem cell niche in the ventral domain of the embryonic dorsal aorta. Proc Natl Acad Sci U S A 2007;104:9399–403.
66. de Bruijn MF, Ma X, Robin C, Ottersbach K, Sanchez MJ, Dzierzak E. Hematopoietic stem cells localize to the endothelial cell layer in the midgestation mouse aorta. Immunity 2002;16:673–83.
67. Mascarenhas MI, Parker A, Dzierzak E, Ottersbach K. Identification of novel regulators of hematopoietic stem cell development through refinement of stem cell localization and expression profiling. Blood 2009;114:4645–53.

68. North T, Gu TL, Stacy T, et al. Cbfa2 is required for the formation of intra-aortic hematopoietic clusters. Development 1999;126:2563–75.
69. 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;144:2323–37.
70. Cai Z, de Bruijn M, Ma X, et al. Haploinsufficiency of AML1 affects the temporal and spatial generation of hematopoietic stem cells in the mouse embryo. Immunity 2000;13:423–31.
71. 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;9:2517.
72. Li Y, Gao L, Hadland B, Tan K, Speck NA. CD27 marks murine embryonic hematopoietic stem cells and type II prehematopoietic stem cells. Blood 2017;130:372–6.
73. Vink CS, Calero-Nieto FJ, Wang X, et al. Iterative single-cell analyses define the transcriptome of the first functional hematopoietic stem cells. Cell Rep 2020;31:107627.
74. Porcheri C, Golan O, Calero-Nieto FJ, et al. Notch ligand Dll4 impairs cell recruitment to aortic clusters and limits blood stem cell generation. EMBO J 2020;39:e104270.
75. Sa da Bandeira D, Kilpatrick AM, Marques M, et al. PDGFRbeta(+) cells play a dual role as hematopoietic precursors and niche cells during mouse ontogeny. Cell Rep 2022;40:111114.
76. Pouget C, Gautier R, Teillet MA, Jaffredo T. Somite-derived cells replace ventral aortic hemangioblasts and provide aortic smooth muscle cells of the trunk. Development 2006;133:1013–22.
77. Ottersbach K. Endothelial-to-haematopoietic transition: an update on the process of making blood. Biochem Soc Trans 2019;47:591–601.
78. Thambyrajah R, Bigas A. Notch signaling in HSC emergence: when, why and how. Cells 2022;11:358.
79. Dzierzak E, Bigas A. Blood development: hematopoietic stem cell dependence and independence. Cell Stem Cell 2018;22:639–51.
80. Gao X, Xu C, Asada N, Frenette PS. The hematopoietic stem cell niche: from embryo to adult. Development 2018;145:dev139691.
81. Vink CS, Dzierzak E. The (intra-aortic) hematopoietic cluster cocktail: what is in the mix? Exp Hematol 2023;118:1–11.
82. Vink CS, Popravko A, Dzierzak E. De novo hematopoietic (stem) cell generation - A differentiation or stochastic process? Curr Opin Cell Biol 2023;85:102255.
83. Vink CS, Mariani SA, Dzierzak E. Embryonic Origins of the Hematopoietic System: Hierarchies and Heterogeneity. Hemasphere 2022;6:e737.
84. Rybtsov S, Batsivari A, Bilotkach K, et al. Tracing the origin of the HSC hierarchy reveals an SCF-dependent, IL-3-independent CD43(-) embryonic precursor. Stem Cell Reports 2014;3:489–501.
85. Rybtsov S, Sobiesiak M, Taoudi S, et al. Hierarchical organization and early hematopoietic specification of the developing HSC lineage in the AGM region. J Exp Med 2011;208:1305–15.
86. Taoudi S, Gonneau C, Moore K, et al. Extensive hematopoietic stem cell generation in the AGM region via maturation of VE-cadherin+CD45+ pre-definitive HSCs. Cell Stem Cell 2008;3:99–108.
87. Yokomizo T, Suda T. Development of the hematopoietic system: expanding the concept of hematopoietic stem cell-independent hematopoiesis. Trends Cell Biol 2024;34:161–72.
88. Bertrand JY, Giroux S, Golub R, et al. Characterization of purified intraembryonic hematopoietic stem cells as a tool to define their site of origin. Proc Natl Acad Sci U S A 2005;102:134–9.
89. Chen MJ, Li Y, De Obaldia ME, et al. Erythroid/myeloid progenitors and hematopoietic stem cells originate from distinct populations of endothelial cells. Cell Stem Cell 2011;9:541–52.
90. North TE, de Bruijn MF, Stacy T, et al. Runx1 expression marks long-term repopulating hematopoietic stem cells in the midgestation mouse embryo. Immunity 2002;16:661–72.
91. Garcia-Porrero JA, Manaia A, Jimeno J, Lasky LL, Dieterlen-Lievre F, Godin IE. Antigenic profiles of endothelial and hemopoietic lineages in murine intraembryonic hemogenic sites. Dev Comp Immunol 1998;22:303–19.
92. Roques M, Durand C, Gautier R, et al. Endoglin expression level discriminates long-term hematopoietic from short-term clonogenic progenitor cells in the aorta. Haematologica 2012;97:975–9.
93. Boisset JC, Clapes T, Van Der Linden R, Dzierzak E, Robin C. Integrin alphaIIb (CD41) plays a role in the maintenance of hematopoietic stem cell activity in the mouse embryonic aorta. Biol Open 2013;2:525–32.
94. Gonzalez Galofre ZN, Kilpatrick AM, Marques M, et al. Runx1+ vascular smooth muscle cells are essential for hematopoietic stem and progenitor cell development in vivo. Nat Commun 2024;15:1653.
95. 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;18:21–32.
96. 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;30:376–92.
97. Swiers G, Baumann C, O'Rourke J, et al. Early dynamic fate changes in haemogenic endothelium characterized at the single-cell level. Nat Commun 2013;4:2924.
98. Kaimakis P, de Pater E, Eich C, et al. Functional and molecular characterization of mouse Gata2-independent hematopoietic progenitors. Blood 2016;127:1426–37.
99. Yokomizo T, Ideue T, Morino-Koga S, et al. Independent origins of fetal liver haematopoietic stem and progenitor cells. Nature 2022;609:779–84.
100. Zielke N, Edgar BA. FUCCI sensors: powerful new tools for analysis of cell proliferation. Wiley Interdiscip Rev Dev Biol 2015;4:469–87.
101. Batsivari A, Rybtsov S, Souilhol C, et al. Understanding Hematopoietic Stem Cell Development through Functional Correlation of Their Proliferative Status with the Intra-aortic Cluster Architecture. Stem Cell Reports 2017;8:1549–62.
102. 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;143:1284–9.
103. Eilken HM, Nishikawa S, Schroeder T. Continuous single-cell imaging of blood generation from haemogenic endothelium. Nature 2009;457:896–900.
104. Lancrin C, Sroczynska P, Stephenson C, Allen T, Kouskoff V, Lacaud G. The haemangioblast generates haematopoietic cells through a haemogenic endothelium stage. Nature 2009;457:892–5.
105. Zovein AC, Hofmann JJ, Lynch M, et al. Fate tracing reveals the endothelial origin of hematopoietic stem cells. Cell Stem Cell 2008;3:625–36.
106. 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;457:887–91.
107. de Pater E, Kaimakis P, Vink CS, et al. Gata2 is required for HSC generation and survival. J Exp Med 2013;210:2843–50.
108. Kissa K, Herbomel P. Blood stem cells emerge from aortic endothelium by a novel type of cell transition. Nature 2010;464:112–5.
109. Bertrand JY, Chi NC, Santoso B, Teng S, Stainier DY, Traver D. Haematopoietic stem cells derive directly from aortic endothelium during development. Nature 2010;464:108–11.
110. Lam EY, Hall CJ, Crosier PS, Crosier KE, Flores MV. Live imaging of Runx1 expression in the dorsal aorta tracks the emergence of blood progenitors from endothelial cells. Blood 2010;116:909–14.
111. Lancino M, Majello S, Herbert S, et al. Anisotropic organization of circumferential actomyosin characterizes hematopoietic stem cells emergence in the zebrafish. Elife 2018;7:e37355.
112. Lagendijk AK, Gomez GA, Baek S, et al. Live imaging molecular changes in junctional tension upon VE-cadherin in zebrafish. Nat Commun 2017;8:1402.
113. Sahai-Hernandez P, Pouget C, Eyal S, et al. Dermomyotome-derived endothelial cells migrate to the dorsal aorta to support hematopoietic stem cell emergence. Elife 2023;12:e58300.

114. Nguyen PD, Hollway GE, Sonntag C, et al. Haematopoietic stem cell induction by somite-derived endothelial cells controlled by meox1. Nature 2014;512:314–8.
115. Qiu J, Fan X, Wang Y, et al. Embryonic hematopoiesis in vertebrate somites gives rise to definitive hematopoietic stem cells. J Mol Cell Biol 2016;8:288–301.
116. Poullet N, Golushko I, Lorman V, et al. Mechanical instabilities of aorta drive blood stem cell production: a live study. Cell Mol Life Sci 2020;77:3453–64.
117. Campinho P, Lamperti P, Boselli F, Vilfan A, Vermot J. Blood flow limits endothelial cell extrusion in the. Zebrafish Dorsal Aorta. Cell Rep. 2020;31:107505.
118. Boisset JC, Andrieu-Soler C, van Cappellen WA, Clapes T, Robin C. Ex vivo time-lapse confocal imaging of the mouse embryo aorta. Nat Protoc 2011;6:1792–805.
119. Boisset JC, van Cappellen W, Andrieu-Soler C, Galjart N, Dzierzak E, Robin C. In vivo imaging of haematopoietic cells emerging from the mouse aortic endothelium. Nature 2010;464:116–20.
120. Adamo L, Naveiras O, Wenzel PL, et al. Biomechanical forces promote embryonic haematopoiesis. Nature 2009;459:1131–5.
121. North TE, Goessling W, Peeters M, et al. Hematopoietic stem cell development is dependent on blood flow. Cell 2009;137:736–48.
122. Diaz MF, Li N, Lee HJ, et al. Biomechanical forces promote blood development through prostaglandin E2 and the cAMP-PKA signaling axis. J Exp Med 2015;212:665–80.
123. Horton PD, Dumbali SP, Bhanu KR, Diaz MF, Wenzel PL. Biomechanical regulation of hematopoietic stem cells in the developing embryo. Curr Tissue Microenviron Rep 2021;2:1–15.
124. Ganuza M, Hall T, Finkelstein D, Chabot A, Kang G, McKinney-Freeman S. Lifelong haematopoiesis is established by hundreds of precursors throughout mammalian ontogeny. Nat Cell Biol 2017;19:1153–63.
125. Eich C, Arlt J, Vink CS, et al. In vivo single cell analysis reveals Gata2 dynamics in cells transitioning to hematopoietic fate. J Exp Med 2018;215:233–48.
126. Jaffredo T, Gautier R, Brajeul V, Dieterlen-Lievre F. Tracing the progeny of the aortic hemangioblast in the avian embryo. Dev Biol 2000;224:204–14.
127. Frame JM, Fegan KH, Conway SJ, McGrath KE, Palis J. Definitive hematopoiesis in the yolk sac emerges from Wnt-responsive hemogenic endothelium independently of circulation and arterial identity. Stem Cells 2016;34:431–44.
128. 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;2:252–63.
129. Gekas C, Rhodes KE, Van Handel B, Chhabra A, Ueno M, Mikkola HK. Hematopoietic stem cell development in the placenta. Int J Dev Biol 2010;54:1089–98.
130. Serikov V, Hounshell C, Larkin S, et al. Human term placenta as a source of hematopoietic cells. Exp Biol Med (Maywood) 2009;234:813–23.
131. Nakano H, Liu X, Arshi A, et al. Haemogenic endocardium contributes to transient definitive haematopoiesis. Nat Commun 2013;4:1564.
132. Shigeta A, Huang V, Zuo J, et al. Endocardially derived macrophages are essential for valvular remodeling. Dev Cell 2019;48:617–630.e3.
133. Liu K, Jin H, Zhang S, et al. Intercellular genetic tracing of cardiac endothelium in the developing heart. Dev Cell 2023;58:1502–1512.e3.
134. Yvernogeau L, Gautier R, Petit L, et al. In vivo generation of haematopoietic stem/progenitor cells from bone marrow-derived haemogenic endothelium. Nat Cell Biol 2019;21:1334–45.
135. Iizuka K, Yokomizo T, Watanabe N, et al. Lack of phenotypical and morphological evidences of endothelial to hematopoietic transition in the murine embryonic head during hematopoietic stem cell emergence. PLoS One 2016;11:e0156427.
136. Li Z, Vink CS, Mariani SA, Dzierzak E. Subregional localization and characterization of Ly6aGFP-expressing hematopoietic cells in the mouse embryonic head. Dev Biol 2016;416:34–41.
137. Zovein AC, Turlo KA, Ponec RM, et al. Vascular remodeling of the vitelline artery initiates extravascular emergence of hematopoietic clusters. Blood 2010;116:3435–44.
138. Yzaguirre AD, Speck NA. Extravascular endothelial and hematopoietic islands form through multiple pathways in midgestation mouse embryos. Dev Biol 2016;415:111–21.
139. Yokomizo T, Ng CE, Osato M, Dzierzak E. Three-dimensional imaging of whole midgestation murine embryos shows an intravascular localization for all hematopoietic clusters. Blood 2011;117:6132–4.
140. Chalin D, Bureau C, Parmeggiani A, Rochal S, Kissa K, Golushko I. Modeling and live imaging of mechanical instabilities in the zebrafish aorta during hematopoiesis. Sci Rep 2021;11:9316.
141. Campinho P, Vilfan A, Vermot J. Blood Flow Forces in Shaping the Vascular System: A. Focus on Endothelial Cell Behavior. Front Physiol. 2020;11:552.
142. Spasic M, Jacobs CR. Primary cilia: Cell and molecular mechanosensors directing whole tissue function. Semin Cell Dev Biol 2017;71:42–52.
143. Liu Z, Tu H, Kang Y, et al. Primary cilia regulate hematopoietic stem and progenitor cell specification through Notch signaling in zebrafish. Nat Commun 2019;10:1839.
144. Lundin V, Sugden WW, Theodore LN, et al. YAP regulates hematopoietic stem cell formation in response to the biomechanical forces of blood flow. Dev Cell 2020;52:446–460.e5.
145. Lee JS, Yu Q, Shin JT, et al. Klf2 is an essential regulator of vascular hemodynamic forces in vivo. Dev Cell 2006;11:845–57.
146. Parmar KM, Larman HB, Dai G, et al. Integration of flow-dependent endothelial phenotypes by Kruppel-like factor 2. J Clin Invest 2006;116:49–58.
147. Mack JJ, Mosqueiro TS, Archer BJ, et al. NOTCH1 is a mechanosensor in adult arteries. Nat Commun 2017;8:1620.
148. Weijts B, Gutierrez E, Saikin SK, et al. Blood flow-induced Notch activation and endothelial migration enable vascular remodeling in zebrafish embryos. Nat Commun 2018;9:5314.
149. Kobayashi I, Kobayashi-Sun J, Kim AD, et al. Jam1a-Jam2a interactions regulate haematopoietic stem cell fate through Notch signalling. Nature 2014;512:319–23.
150. Espin-Palazon R, Stachura DL, Campbell CA, et al. Proinflammatory signaling regulates hematopoietic stem cell emergence. Cell 2014;159:1070–85.
151. Travnickova J, Tran Chau V, Julien E, et al. Primitive macrophages control HSPC mobilization and definitive haematopoiesis. Nat Commun 2015;6:6227.
152. Theodore LN, Hagedorn EJ, Cortes M, et al. Distinct roles for matrix metalloproteinases 2 and 9 in embryonic hematopoietic stem cell emergence, migration, and niche colonization. Stem Cell Reports 2017;8:1226–41.
153. Mariani SA, Li Z, Rice S, et al. Pro-inflammatory aorta-associated macrophages are involved in embryonic development of hematopoietic stem cells. Immunity 2019;50:1439–1452.e5.
154. Murayama E, Kissa K, Zapata A, et al. Tracing hematopoietic precursor migration to successive hematopoietic organs during zebrafish development. Immunity 2006;25:963–75.
155. Tamplin OJ, Durand EM, Carr LA, et al. Hematopoietic stem cell arrival triggers dynamic remodeling of the perivascular niche. Cell 2015;160:241–52.
156. Xue Y, Lv J, Zhang C, Wang L, Ma D, Liu F. The vascular niche regulates hematopoietic stem and progenitor cell lodgment and expansion via klf6a-ccl25b. Dev Cell 2017;42:349–362.e4.
157. Mahony CB, Fish RJ, Pasche C, Bertrand JY. tfec controls the hematopoietic stem cell vascular niche during zebrafish embryogenesis. Blood 2016;128:1336–45.

158. Blaser BW, Moore JL, Hagedorn EJ, et al. CXCR1 remodels the vascular niche to promote hematopoietic stem and progenitor cell engraftment. J Exp Med 2017;214:1011–27.
159. Binder V, Li W, Faisal M, et al. Microenvironmental control of hematopoietic stem cell fate via CXCL8 and protein kinase C. Cell Rep 2023;42:112528.
160. Xia J, Kang Z, Xue Y, et al. A single-cell resolution developmental atlas of hematopoietic stem and progenitor cell expansion in zebrafish. Proc Natl Acad Sci U S A 2021;118:e2015748118.
161. Li D, Xue W, Li M, et al. VCAM-1(+) macrophages guide the homing of HSPCs to a vascular niche. Nature 2018;564:119–24.
162. Wattrus SJ, Smith ML, Rodrigues CP, et al. Quality assurance of hematopoietic stem cells by macrophages determines stem cell clonality. Science 2022;377:1413–9.
163. Hagedorn EJ, Perlin JR, Freeman RJ, et al. Transcription factor induction of vascular blood stem cell niches in vivo. Dev Cell 2023;58:1037–1051.e4.
164. Agarwala S, Kim KY, Phan S, et al. Defining the ultrastructure of the hematopoietic stem cell niche by correlative light and electron microscopy. Elife 2022;11:e64835.
165. Suster ML, Kikuta H, Urasaki A, Asakawa K, Kawakami K. Transgenesis in zebrafish with the tol2 transposon system. Methods Mol Biol 2009;561:41–63.
166. Akitake CM, Macurak M, Halpern ME, Goll MG. Transgenerational analysis of transcriptional silencing in zebrafish. Dev Biol 2011;352:191–201.
167. Kazenwadel J, Venugopal P, Oszmiana A, et al. A Prox1 enhancer represses haematopoiesis in the lymphatic vasculature. Nature 2023;614:343–8.
168. Ulloa BA, Habbsa SS, Potts KS, et al. Definitive hematopoietic stem cells minimally contribute to embryonic hematopoiesis. Cell Rep 2021;36:109703.
169. Gao S, Zhang Y, Liu F. Revisiting the lineage contribution of hematopoietic stem and progenitor cells. Development 2023;150:dev201609.
170. Frenz-Wiessner S, Fairley SD, Buser M, et al. Generation of complex bone marrow organoids from human induced pluripotent stem cells. Nat Methods 2024;21:868–81.
171. Li Y, Nie Y, Yang X, et al. Integration of Kupffer cells into human iPSC-derived liver organoids for modeling liver dysfunction in sepsis. Cell Rep 2024;43:113918.
172. Wimmer RA, Leopoldi A, Aichinger M, Kerjaschki D, Penninger JM. Generation of blood vessel organoids from human pluripotent stem cells. Nat Protoc 2019;14:3082–100.
173. van den Brink SC, van Oudenaarden A. 3D gastruloids: a novel frontier in stem cell-based in vitro modeling of mammalian gastrulation. Trends Cell Biol 2021;31:747–59.
174. Sugimura R, Ohta R, Mori C, et al. Biomimetic aorta-gonad-Mesonephros-on-a-Chip to study human developmental hematopoiesis. Biomed Microdevices 2020;22:34.
175. Xiong F, Megason SG. Abstracting the principles of development using imaging and modeling. Integr Biol (Camb) 2015;7:633–42.