Cell Regeneration

CSCB
CHINESE SOCIETY FOR CELL BIOLOGY

REVIEW   **Open Access**

Zebrafish: a convenient tool for myelopoiesis research

Check for updates

Yang-Xi Hu[1] and Qing Jing[2]*

Abstract

Myelopoiesis is the process in which the mature myeloid cells, including monocytes/macrophages and granulocytes, are developed. Irregular myelopoiesis may cause and deteriorate a variety of hematopoietic malignancies such as leukemia. Myeloid cells and their precursors are difficult to capture in circulation, let alone observe them in real time. For decades, researchers had to face these difficulties, particularly in in-vivo studies. As a unique animal model, zebrafish possesses numerous advantages like body transparency and convenient genetic manipulation, which is very suitable in myelopoiesis research. Here we review current knowledge on the origin and regulation of myeloid development and how zebrafish models were applied in these studies.

Keywords: Myelopoiesis, Hematopoietic stem/progenitor cells, Myeloid disorder, Zebrafish

Background

Myelopoiesis is a complex process in which the myeloid progenitors, with multiple differentiation events, develop into mature myeloid cells, including monocytes/macrophages and granulocytes. The myeloid progenitor cells derive from hematopoietic stem cells (HSCs), the pluripotent adult stem cells with self-renewal and differentiation potential as well as the origin of mature blood cells in the body. The characteristics of myeloid progenitor cells (MPCs) and HSCs, such as rare number, complex differentiation pedigree, and uncertain migration in the circulation, greatly increase the difficulty of in vivo studies using conventional animal models.

Zebrafish emerged as a classical developmental and embryological model organism because of its transparent in vitro fertilization and rapid embryonic development. Thousands of zebrafish mutants with abnormal hematopoiesis were produced through forward genetic screening, showing a high degree of conservation of pathogenic genes in the process of hematopoiesis in different species (Gore et al. 2018). The high conservation of zebrafish genes provides easy access to identify genes related to the hematopoietic disorders of human beings (Robertson et al. 2016). Through different genetic means and drug screening, the expression of specific genes could be induced or inhibited in specific tissues of zebrafish, paving the way to precisely study their roles in human diseases (Kafina and Paw 2018). Fortunately, zebrafish embryos can tolerate severe anemia, myeloproliferative diseases, and other serious hematopoietic diseases that usually lead to embryonic death of mammalian models to a large extent, making it indispensable in the in vivo research of human hematopoiesis and hematological malignancies. Through reverse genetic editing, thousands of zebrafish embryos can be genetically manipulated to test the gene mutations found in human research, even in a single experiment. Through the transient overexpression of homologous genes related to human hematopoietic malignancies in zebrafish, or the construction of stable transgenic lines of these genes, the studies of acute and chronic hematopoietic diseases have been greatly facilitated.

Zebrafish has the basic myeloid cell types observed in mammals, including monocyte, neutrophil, eosinophil,

*Correspondence: qjing@sibs.ac.cn
[2] CAS Key Laboratory of Tissue Microenvironment and Tumor, Shanghai Institute of Nutrition and Health, Chinese Academy of Sciences, 320 Yue-Yang Road, Shanghai 200031, China
Full list of author information is available at the end of the article

mast cell, dendritic cell, and so on. The myelopoiesis process is also conserved between zebrafish and most other vertebrates. Thus, the findings from zebrafish models are possible to be directly applied to other higher vertebrates (Zang et al. 2022). At present, researchers constructed transgenic lines in zebrafish that labeled specific cell clusters to track the generation and migration of MPCs and myeloid cells during the development and under various chemical or genetic disturbances in real time. In this review, we provide an overview of the myelopoiesis research using zebrafish models and their findings.

The myelopoiesis of zebrafish

Figure 1 depicts the process of zebrafish myelopoiesis and some of the recently acknowledged regulators. The primitive hematopoiesis of zebrafish begins at 11 hours post fertilization (hpf) and mainly produces primitive macrophages, neutrophils, and erythrocytes (Detrich 3rd et al. 1995; Le Guyader et al. 2008). But primitive myeloid cells and erythrocytes have different developmental origins. The anterior lateral mesoderm (ALM) locates a kind of precursor cells with the dual potential to generate hematopoietic cells, and vascular endothelial cells (ECs) called hemangioblasts (Vogeli et al. 2006). Single-cell fate tracing of late blastocysts and gastrula of zebrafish proved that individual hemangioblasts could produce hematopoietic cells and ECs at the same time (Vogeli et al. 2006). Etv2/etsrp, an ETS transcription factor, is a key regulator of hemangioblasts as well as one of the earliest markers for the progenitors of ECs and hematopoietic cells (Craig et al. 2015; Sumanas and Lin 2006). At around 12 hpf, the myeloid precursors, the origin of primitive macrophages, are differentiated from hemangioblasts. Subsequently, myeloid precursors begin to migrate and differentiate into macrophages and granulocytes in the rostral blood island (RBI) (Bennett et al. 2001). Primitive hematopoietic cells produce macrophages and neutrophils that initiate innate immunity. Primitive neutrophils have little phagocytic capacity yet response to infection or environmental stress (Le Guyader et al. 2008). The differentiation of hemangioblast into myeloid precursor is regulated by multiple factors and signaling pathways. For instance, the *FGF/MAPK* signal pathway (Faloon et al. 2000), the *VEGF/Flk1* signal pathway (Cao and Yao 2011), and the *BMP* signal pathway (Kennedy et al. 2007) are important for the development and differentiation of hemangioblasts. Dysfunction

of the key components in these pathways will lead to the failure of HPSC generation and affect the expression of hematopoietic marker genes such as *scl*, *gfi1*, and *lmo2*.

The definitive hematopoiesis could be subdivided into two waves. At about 24 hpf, there is a transient hematopoietic wave in the posterior blood island (PBI) to produce erythroid-myeloid progenitors (EMPs). These cells do not have the ability of self-renewal and can only differentiate into myeloid and erythroid blood cells (Bertrand et al. 2007). At 28-30 hpf, the second wave of the definitive hematopoietic process begins to occur (Bertrand et al. 2007). Different from the transient wave, the definitive wave sustains into the adult stage of zebrafish, producing HSCs, which differentiate into lymphocytes, myeloid cells, and erythroid cells. In the ventral wall of the dorsal aorta (VDA), which is homologous to the aorta-gonad-mesonephros (AGM) region of mammals, hemogenic endothelial cells (HECs) differentiate into HSCs through the process of endothelial hematopoietic transition (EHT) (Kissa and Herbomel 2010). The transcription factor c-myb is one of the most important regulators of myelopoiesis, whose inhibition will result in impaired egression of HSPC from the VDA while hyperactivity leads to abnormal granulocyte expansion (Liu et al. 2017; Zhang et al. 2011). Nascent HSCs robustly regenerate after selective injury, yet do not contribute to early lymphopoiesis and myelopoiesis. Instead, HSC-independent blood progenitors give rise to embryonic lymphomyelopoiesis (Ulloa et al. 2021). From 48 hpf, *c-myb* positive cells can be detected in the caudal hematopoietic tissue (CHT), which is functionally homologous to mammalian fetal liver and contributes to the growth and differentiation of HSCs (Murayama et al. 2006). Through real-time imaging, it was observed that HSCs migrated to the CHT through circulation, after which they sprouted out and stayed in the microenvironment around ECs (Tamplin et al. 2015). After close contact with ECs, HSCs divide in an asymmetric manner, which seems to depend on the cellular niche of mesenchymal stem cells (MSCs) (Morrison and Kimble 2006; Morrison and Spradling 2008). When the embryo develops to 4 dpf, HSCs gradually migrate to the kidneys and begin to settle down. The kidney is equivalent to the bone marrow of mammals and becomes a definitive hematopoietic organ, maintaining the whole blood system and producing blood cells required for the whole life stage, including myeloid cells (Thompson et al. 1998). In the process of

(See figure on next page.)

Fig. 1 Zebrafish myelopoiesis and recently acknowledged regulators. Zebrafish myeloid cells derives from myeloid progenitor cells and hematopoietic stem cells. The sites each hematopoietic wave takes place are indicated together with timelines, as are some of the recently reported regulators of zebrafish myelopoiesis. The promotive and suppressive regulators are labelled in red and blue, respectively. RBI, rostral blood island; ALM, anterior lateral mesoderm; EC, endothelial cell; MPC, myeloid progenitor cell; PBI, post blood island; EMP, erythroid-myeloid progenitor; DA, dorsal aorta; CHT, caudal hematopoietic tissue; HEC, hemogenic endothelial cell; HSC, hematopoietic stem cell; CMP, common myeloid progenitor
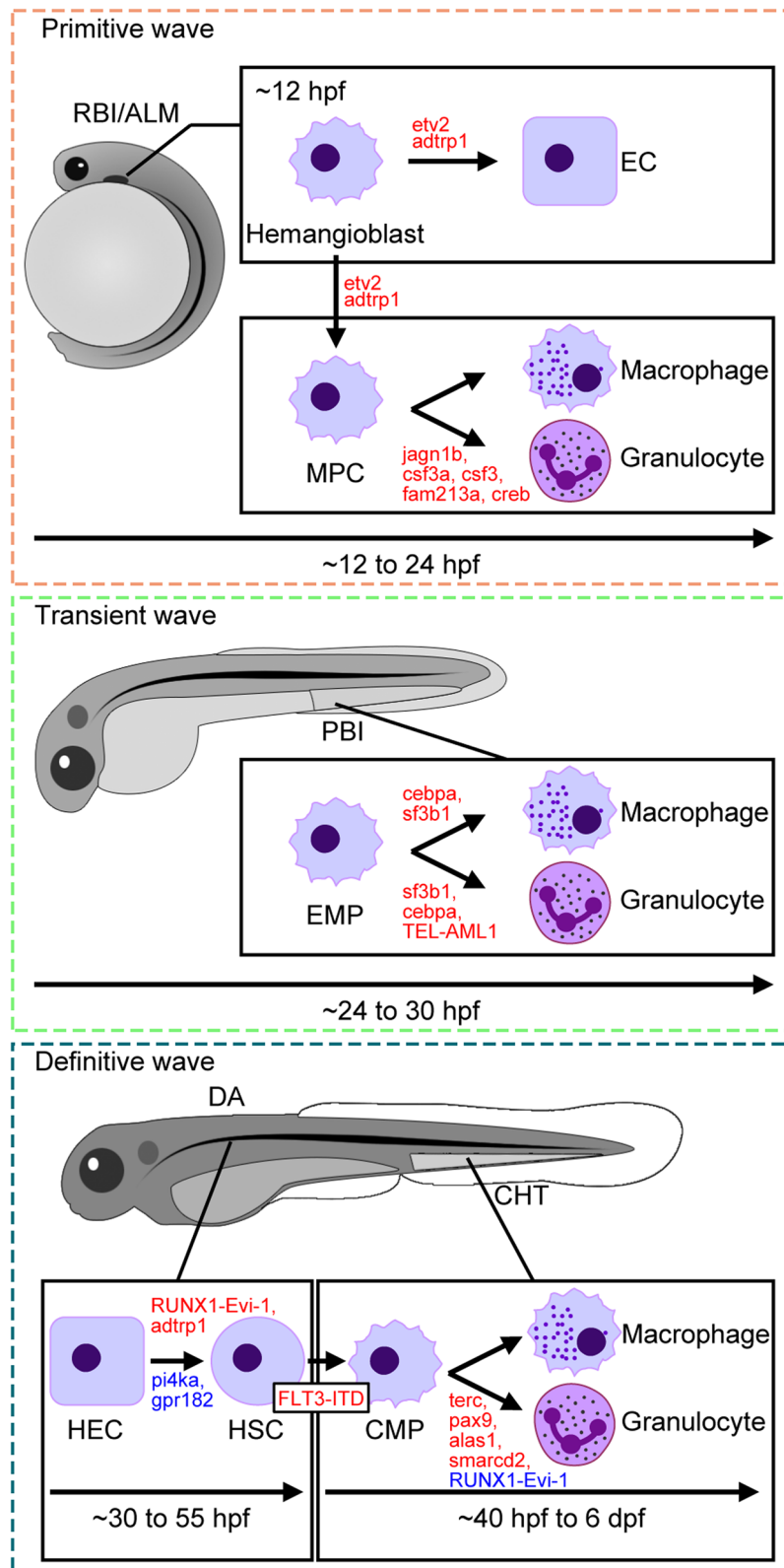
Fig. 1 (See legend on previous page.)

myelopoiesis, a part of macrophages migrates to specific tissue and then specializes into resident macrophages. The origin, function, and fate of various resident macrophages are yet elusive. Microglia, the CNS resident macrophages, is once supposed to originate from a single source, the yolk sac. Using light-induced high temporal-spatial resolution fate mapping, Xu et al. revealed that in zebrafish, the embryonic/larval microglia arise from the RBI, whereas the adult microglia arise from the VDA, regulated by *runx1* but not *c-myb*, which is different from the embryonic/larval microglia (Xu et al. 2015).

Besides developmental myelopoiesis, there exists emergency myelopoiesis, which generates new neutrophils and other myeloid cells following infection or inflammation (Basu et al. 2000; Ueda et al. 2009). The emergency myelopoiesis is associated with the expansion of HSPCs and MPCs. To some extent, HSPC differentiation could be enhanced by infective or inflammatory cues. However, excessive burden causes the apoptosis of HSPCs and therefore suppresses emergency myelopoiesis (Abidin et al. 2017). As to be described in detail below, the transcription factor pu.1 plays a main promotive role in physiological macrophage development (Hou et al. 2016). However, in emergency monopoiesis, pu.1 is suppressive, which may partly explain the different patterns of emergency myelopoiesis in different scenarios. In emergency myelopoiesis, some proinflammatory cytokines regulate various hematopoietic compartments. For instance, IFN-γ is known to negatively regulate HSC proliferation until recent studies discovered that IFN-γ also promotes HSC formation and consequent myelopoiesis (Qin and Zhang 2017). Macrophage-derived il1β actives nf-κb and c/ebpβ, two fast-reactive transcription factors, rapidly. In an LPS-induced systemic infection zebrafish model, the macrophage is the first cell type to detect LPS and secret il1β, which is further responded to by MPCs, rather than HSPCs, expanding myelopoiesis through the synergistic cooperation of nf-κb and c/ebpβ (Wei et al. 2020).

Using zebrafish to explore the regulation of myelopoiesis

Zebrafish myelopoiesis is complicated yet precisely regulated by both genetic and extrinsic factors, as described in a precious review before (Xu et al. 2012). Firstly, the specification and differentiation of myeloid precursors and MPCs are controlled by various transcription factors, namely *runx1*, *pu.1*, *irf8*, *gata* family, *lmo2*, *scl*, and others in a spatially and temporally coordinated manner. Table 1 summarizes some of the important transcription factors in myelopoiesis and myeloid disorders. In addition, the regulatory factors upstream these transcription factors, such as *tif1g*, *bik1f*, and *gfi1.1*, are also found effective in myeloid-erythroid lineage choice of primitive hematopoietic progenitors. Secondly, complex signaling pathways regulate the differentiation of hemangioblast into hematopoietic cells, including the *cloche/etsrp/scl* pathway and the *Bmp/Alk8* pathway. Thirdly, extrinsic factors like granulocyte colony-stimulating factor (GCSF) affect the development and migration of myeloid cells (Meier et al. 2022). Given these known, in this section, we selected and reviewed several recent progresses in the understanding of myelopoiesis gained from zebrafish models.

Table 1 Important transcription factors in myelopoiesis and myeloid disorders

Transcription factor	Family	Genetic manipulation	Hematopoietic phenotype	Reference
pu.1	ETS	Knockout	Immature granulocytes	(Sun et al. 2013)
spi-b	ETS	Knockdown	Decreased neutrophils and macrophages	(Bukrinsky et al. 2009)
runx1	Runt	Knockout	Immature neutrophils	(Huang et al. 2021)
C/EBPα	Runt	Knockout	Decreased macrophages and neutrophils	(Dai et al. 2016; Hockings et al. 2018)
C/EBPβ	Runt	Knockdown	Suppressed emergency granulopoiesis	(Hall et al. 2012)
C/EBPδ	Runt	Knockdown	Increased myeloid differentiation	(Tregnago et al. 2016)
irf8	IRF	Knockout	Impaired macrophage specification and longevity	(Tamura et al. 2005)
gata1	GATA	Knockout	Reduced eosinophils, morphologically abnormal mast cells, impaired dendritic-cell differentiation	(Gutiérrez et al. 2007; Migliaccio et al. 2003; Yu et al. 2002)
gata2a	GATA	Knockout	Reduced primitive monocyte/macrophages	(Peña et al. 2021)
gata2b	GATA	Knockout	Reduced definitive HSPCs and neutrophils	(Gioacchino et al. 2021; Peña et al. 2021)
gata4/5/6	GATA	Knockdown	Reduced definitive granulocytes and macrophages	(Jia et al. 2019)
SOX4	HMG	Overexpression	Enhance MPC proliferation	(Sandoval et al. 2012)
pax9	Pax	Knockout	Reduced neutrophils	(Pak et al. 2021)
scl	HLH	Knockdown	Impaired monocytes and macrophages	(Dooley et al. 2005)
lmo2	LIM	Knockdown	Reduced granulocytes and macrophages	(Patterson et al. 2007)
c-myb	Myb	Knockout	Immature neutrophils	(Jin et al. 2016)

Transcriptions factors and their regulation

The well-known hematopoietic transcription factor runx1 regulates neutrophil maturation in zebrafish primitive myelopoiesis. Huang et al. reported that runx1, synergistically with another hematopoietic transcription factor, c-myb, controls myelopoiesis through activating downstream genes involving neutrophil maturation (Huang et al. 2021). In definitive hematopoiesis, runx1 shows a dynamic expression pattern during the specialization of HSCs. In zebrafish, *runx1* deficiency will cause the termination of EHT, and immature HSCs will be rapidly broken into fragments (Kissa and Herbomel 2010). When the cofactor cbfβ of runx1 is knocked out, HSCs cannot move out from the dorsal aorta (DA), and definitive hematopoiesis cannot occur (Bresciani et al. 2014). In three new zebrafish lines with *runx1* deficiency, Bresciani et al. observed that runx1 and gata2 act redundant roles for HSC production acting as each other's safeguard (Bresciani et al. 2021).

The CCAAT/enhancer-binding proteins (C/EBPs) are a group of transcription factors that play important roles in developmental myelopoiesis conserved between zebrafish and other mammals. C/EBPα maintains the steady-state granulopoiesis while C/EBPβ is required for stress-induced granulopoiesis. Hockings et al. reported that biallelic mutations in both C and N terminals of c/ebpα caused severely impaired myelocyte and monocyte maturation (Hockings et al. 2018; Hockings et al. 2016). Dai et al. reported that c/ebpα-deficient zebrafish mutants manifested a lack of macrophages and neutrophils, which is caused by the cell cycle arrest of MPCs. They found that the function of c/ebpα on myelopoiesis probably requires the corporation with runx1 (Dai et al. 2016). C/EBPβ is indispensable for emergency granulopoiesis (Hall et al. 2012) and responsible for expanded myelopoiesis in many hematological malignancies like chronic myeloid leukemia (CML) (Hirai et al. 2015). C/EBPδ expression increased in a zebrafish model overexpressing CREB, a proto-oncogene frequently overexpressed in AML patients, in the myeloid lineage, causing differentiation arrest of myelopoiesis (Tregnago et al. 2016). C-myb is one of the earliest transcription factors expressed in HSCs, whose loss-of-function leads to severe defects in neutrophil differentiation in zebrafish. C-myb synergistically functions with cebp1, the zebrafish functional homolog of mammalian C/EBPε, directly targets *lyz* (encodes a key protein component in neutrophils), thereby promoting the maturation of neutrophils (Jin et al. 2016). PTEN, a famous tumor suppressor, regulates definitive myelopoiesis in zebrafish via promoting *c/ebpα* and suppressing the *PI3K/mTOR* pathway. *Pten*-deficient zebrafish larvae manifest reduced apoptosis and strongly expansive definitive myelopoiesis while the maturation and immune response of myeloid cells are blocked (Dong et al. 2014).

Pu.1 is an essential ETS-family transcription factor for generating myeloid precursors and their fate determination during myelopoiesis. The suppression of *pu.1* results in increased immature myeloid cells (Sun et al. 2013), while its upregulation induced by various factors such as histone demethylase Jmjd3 leads to enhanced myeloid commitment and repressed erythroid commitment during both the primitive and definitive myelopoiesis (Yu et al. 2018). Even though it is well known that *pu.1* is indispensable in myelopoiesis, whether it is differentially required for the primitive and definitive stages remains unknown. In zebrafish, Yu et al. noticed that the VDA-derived macrophage development depends on the synergistic function of *pu.1* and *spi-b* (another ETS transcription factor). While in the RBI-derived macrophage development, *pu.1* acts upstream *spi-b*. In both waves of macrophage development, irf8 serves as the common downstream factor (Yu et al. 2017). The determination of myeloid fates in HSCs depends mainly on the cross-inhibitory relationship between *gata1* and *pu.1*. Inflammasome mediates the cleavage of *gata1*, not only suppresses erythropoiesis (De Maria et al. 1999), but also shifts the balance of *gata1* and *pu.1*, in turn, regulates myelopoiesis in zebrafish (Tyrkalska et al. 2019). The antagonist-induced inhibition of inflammasomes impaired both physiological and demand-driven myelopoiesis and rescued the phenotypes in zebrafish neutrophilic dermatosis and Diamond-Blackfan anemia models (Tyrkalska et al. 2019).

Interferon regulatory factor 8 (irf8) is an evolutionarily conserved transcription factor differently required in myelopoiesis at different developmental stages (Holtschke et al. 1996; Tamura et al. 2005). Irf8 is essential for macrophage development during primitive and transient definitive hematopoiesis, but not necessary in definitive myelopoiesis starting at 5-6 dpf. Irf8-deficient zebrafish larvae have more neutrophils and excessive cell death in *pu.1*-expressing myeloid cells, indicating that irf8 is critical to macrophage specification and longevity. Interestingly, besides the reduction in number and immaturities, tissue-resident macrophages become apparent in irf8 mutants along with the development, except microglia (Shiau et al. 2015). The interferon regulatory factor 2 binding protein 2a (irf2bp2a), a ring finger protein, regulates neutrophil differentiation in zebrafish, functioning as a ubiquitin E3 ligase. Gao et al. reported that irf2bp2a targets *gfi1aa* for proteasomal degradation, while its mRNA expression is, in turn, passively regulated by gfi1aa. These form a negative feedback loop in zebrafish myelopoiesis (Gao et al. 2021). The interferon regulatory factor 2 binding protein 2b (irf2bp2b) is a

negative transcription regulator. During the definitive myelopoiesis in zebrafish, irf2bp2b influences the fate determination of MPCs in favor of macrophages, under the control of C/EBPα (Wang et al. 2020).

GATA transcription factors comprise a family of zinc finger proteins that bind the consensus DNA sequence (T/A)GATA(A/G). Among them, GATA1 is considered as a key regulator of erythropoiesis. Interestingly, GATA1 is also a regulator of myelopoiesis. For example, the GATA1 loss-of-function was found to cause the reduction of eosinophils. GATA1 deficiency was also reported to induce the morphological abnormality of mast cells and impaired differentiation of dendritic cells. In addition, an acquired N-terminal truncating mutation in GATA1 during fetal life has been reported to cause a preleukaemic condition known as transient abnormal myelopoiesis (TAM) (Garnett et al. 2020). In fact, GATA1 and PU.1 regulate erythropoiesis versus myelopoiesis by antagonizing each other (Campbell et al. 2021). Zebrafish express two *gata2* homologous genes, *gata2a* and *gata2b. Gata2a* began to express in hemangioblasts at 11 hpf, while *gata2b* was specifically expressed in HECs and was crucial to the production of HSCs (Butko et al. 2015). *Gata2a* is vital for primitive myelopoiesis, while *Gata2b* mainly supports definitive myelopoiesis, particularly the development of neutrophils (Peña et al. 2021). *Gata2b* mutants have impaired myeloid lineage differentiation raised in HSPCs. *Gata2b*-deficient HSPCs showed impaired myeloid transcriptional program and increased expression of lymphoid genes (Gioacchino et al. 2021). In addition, gata4/5/6 are all reported to promote primitive myelopoiesis via downregulating miR-210-5p expression (Jia et al. 2019).

SOX4 is known as a transcription factor that belongs to the high-mobility group (HMG) domain superfamily. SOX4 is required in lymphopoiesis (Mallampati et al. 2014) and is reported to be expressed and contributive to the development of multipotent progenitors (Zhang et al. 2014). Yet, how SOX4 affects myelopoiesis remains elusive. Boyd et al. reveal that SOX4 in the murine myeloid cell line 32Dcl3 blocks cytokine-induced granulocyte maturation (Boyd et al. 2006). Through direct binding to the promoter of *CREB* and inducing *CREB* expression, SOX4 up-regulates the target genes of CREB and promotes the proliferation and self-renewal of MPCs (Sandoval et al. 2012). Lu et al. established a transgenic zebrafish model overexpressing human *SOX4* in the myeloid lineage. The mutants developed expanded myelopoiesis with dedifferentiation in kidney marrow at 5 months of age and severely distorted kidney structure with excessive MPCs (Lu et al. 2017).

Paired Box (Pax) transcription regulator family members are involved in many diseases related to organ development processes. Recently, one of its members, Pax9, was found to be indispensable for granulopoiesis without affecting erythropoiesis in zebrafish. *Pax9*-deficiency led to decreased neutrophils and the expression of neutrophil-specific markers in both developmental and emergency granulopoiesis (Pak et al. 2021).

Chromatin regulators

The SMARCD2, also known as BAF60b, is a component of the SWI/SNF complex in HSCs and other hematopoietic cells and regulates the differentiation of myeloid-erythroid progenitor cells (Schim van der Loeff et al. 2021; Witzel et al. 2017). In vivo experiments using zebrafish and mice proved that SMARCD2 is required in neutrophil differentiation, with high evolutionary conservation. SMARCD2 recruits C/EBPε, which further targets the promoters of many granule genes such as *CAMP* and *SERPINA1*, lactoferrin (*LTF*), and matrix metalloproteinase 8/neutrophil collagenase (*MMP8*). Notably, SMARCD2 takes stage-specific roles in granulopoiesis. During granulosis, neutrophils pass through various developmental stages including $CD45^+Lin^-Sca-1^+c-Kit^+$ LSK cells, $CD45^+Lin^-Sca-1^-c-Kit^+CD34^+CD16/32$ $(FCGR)^{int}$ common myeloid progenitors (CMPs), and $CD45^+Lin^-Sca-1^-c-Kit^+CD34^+CD16/32$ $(FCGR)^{high}$ granulocyte–macrophage progenitors (GMPs) or $CD45^+Lin^-Sca-1^-c-Kit^+CD34^-CD16/32$ $(FCGR)^{low}$ megakaryocyte–erythroid progenitors (MEPs). As reported by Witzel et al., SMARCD2 acts as a transcriptional suppressor in immature neutrophils (LSK and CMP cells), while it plays a transcriptional activator in further differentiated stages (MEP and GMP cells) (Witzel et al. 2017).

Ring finger protein 4 (RNF4) also takes part in the regulation of granulopoiesis. The neutrophils were largely decreased in rnf4-deficient zebrafish larvae during both primitive and definitive myelopoiesis. Mechanistically, the hypermethylation of c/ebpα promoter by SUMOylated DNA methyltransferase 1 (DNMT1) led to granulopoiesis defect in rnf4-deficient zebrafish (Wang et al. 2018a). Besides DNMT1, another methyltransferase, smyd5, suppresses the excessive expressions of primitive and definitive myelopoietic genes, such as *pu.1*, *mpx*, *l-plastin*, and *c-myb* (Fujii et al. 2016).

Extrinsic factors and their regulation

The GCSF drives the proliferation and differentiation of granulocytes, monocytes, and macrophages from HSPCs. The two GCSF ligands in zebrafish, gcsfa and gcsfb, are less similar in sequences while closely resemble each other in predicted ligand/receptor interaction sites and structures. Both bind to the GCSF receptor and promote primitive and definitive development of myeloid

cells, and both support the specification and proliferation of HSCs (Stachura et al. 2013). Colony-stimulating factor 1 receptor (Csf1r) also regulates microglia development in vertebrates. Csf1r, or Fms, together with pu.1, function synergistically to support the development of osteoclasts and myeloid cells (Liu et al. 2020). Among them, pu.1 masters osteoclastogenesis, whereas Fms promotes osteoclast maturation. The two zebrafish *csf1r* paralogous genes, *csf1ra* and *csf1rb*, play different roles in myelopoiesis. Ferrero et al. reported that csf1ra, but not csf1rb, is essential for primitive myelopoiesis in zebrafish (Ferrero et al. 2021). For the HSC-derived myelopoiesis in definitive and adult hematopoiesis, it is promoted by csf1rb with reduced macrophages, including microglia (Ferrero et al. 2021). Single-cell RNA sequencing analysis for adult whole kidney marrow (WKM) hematopoietic cells also revealed that *csf1rb* is expressed mainly by MPCs, in a nonoverlapping pattern with *csf1ra* (Hason et al. 2022).

Bone morphogenetic proteins (BMPs) are a group of growth factors regulating HSC differentiation and myelopoiesis. The receptor type Smad proteins, namely Smad1, Smad5, and Smad9, help to transduce nuclear BMP signals. Among them, transcription initiations of smad1 and smad9 are mutually repressive yet indispensable in myelopoiesis, while both are direct downstream of Smad5 (Wei et al. 2014).

The suppressors of cytokine signaling (SOCS) family consists of eight proteins that antagonize the signaling of STAT proteins, whose members are reported by multiple studies to regulate the process of developmental myelopoiesis and progression of myeloid leukemia. The zebrafish model overexpressing SOCS1 in MPCs demonstrated effaced and distorted kidney or spleen structure with increased MPCs and myelopoiesis in kidney marrow (Hou et al. 2017). Among all SOCS family members, socs3b is the most expressed in neutrophils. During 35-48 hpf, the time window for granulation, *socs3b* was usually downregulated, indicating socs3b's regulatory role in myelopoiesis (Banks et al. 2021). The expression of *socs3b* could be upregulated in neutrophils with compound *tet2/3* mutant, indicating that zebrafish tet enzymes demethylate and destabilize *socs3b* mRNA during granulation, thereby maintaining the cytokine signaling to support physiological neutrophil maturation (Banks et al. 2021).

Stag1 and Stag2, two components of cohesin multiprotein complex, corporately regulate the production of hemangioblasts. The depletion of both *stag1a* and *stag2b* in zebrafish results in erythropenia in primitive hematopoiesis. Homozygous loss of *stag1a* leads to expanded LPM with increased *scl*-positive cells and increased *pu.1*-positive cells, indicating the skewing toward primitive myelopoiesis (Ketharnathan et al. 2020).

The mutations of isocitrate dehydrogenase 1/2 (IDH1/2) have been identified in ~30% of cytogenetically normal AML patients. These two enzymes are involved in the citric acid cycle in intermediary metabolism. Zebrafish model carrying human IDH1-R132H mutation, which is frequently identified in AML patients, also developed AML-like phenotypes (Shi et al. 2015). Shi et al. found that zebrafish *idh1* deficiency induced blocked myeloid differentiation, characterized by the increased expression of *pu.1* and decreased expressions of *mpo*, *l-plastin,* and *mpeg1.* Furthermore, *idh1* deficiency led to the significant reduction of *runx1* and *c-myb* expression in the VDA and the CHT region at definitive hematopoiesis. Meanwhile, the supplement of *idh2* mRNA failed to rescue the impaired myeloid differentiation induced by *idh1* deficiency, which means there exists no redundancy between the effects of idh1/2 on myelopoiesis (Shi et al. 2015).

G protein-coupled receptor and their ligands play multifunctional roles in myelopoiesis and erythropoiesis. For example, the deficiency of a G-protein-coupled ADP receptor p2y12 was reported to result in excessive primitive erythropoiesis in zebrafish embryos, mainly attributed to enhanced expression of *gata1* (Li et al. 2021). Another two G-protein-coupled receptors, Gpr56 and Gpr97, were redundantly functioning in normal HSC development and differentiation (Maglitto et al. 2021). Expressed in HECs, the orphan G-protein coupled receptor 182 (Gpr182) suppressed the excessive definitive myelopoiesis by regulating the LTB4 biosynthesis pathway (Kwon et al. 2020).

The 5-aminolevulinate synthase 1 (ALAS1) is a rate-limiting enzyme of the biosynthesis of heme (iron protoporphyrin IX, a prosthetic group on hemoproteins like hemoglobin and myoglobin). Another isoform, ALAS2, mainly expressed in erythroid cells, regulating the production of heme for hemoglobin synthesis. ALAS1 ubiquitously expressed in various cell lineages including neutrophils, implying that heme not only is indispensable in erythropoiesis but also may affect myelopoiesis. The deficiency of *alas1* in zebrafish, which leads to reduced production of heme, impairs the maturation of neutrophils (Lian et al. 2018). This finding also indicates the existence of an intricate crosstalk exists between erythropoiesis and myelopoiesis.

Tissue factor (TF) and the TF pathway have long been known to regulate hematopoiesis. *Adtrp* encodes a regulating protein for the inhibitor of the androgen-dependent TF pathway (TFPI). Recently, a novel *adtrp1-tfpi* axis has been identified to regulate the processes of both primitive and definitive myelopoiesis. Among the two paralogs of *adtrp* in zebrafish (*adtrp1* and *adtrp2*), *adtrp1* promotes the expression of primitive

myelopoietic markers like *pu.1*, *mpo*, and *l-plastin*, and supports HSC specification in definitive myelopoiesis (Wang et al. 2018b).

Noncoding RNAs

Noncoding RNAs are emerging regulatory factors for myelopoiesis, including lncRNAs (Qiu et al. 2021) and circRNAs (Dostalova Merkerova et al. 2020). In addition, many microRNAs have been found to be regulators or effectors of myelopoietic transcription factors (Kim et al. 2019). An example in zebrafish is that the depletion of miR-462/miR-731 decreased erythroid cell number while increased myeloid cell expansion at 48 hpf, indicating their regulatory role in myeloid-erythroid differentiation. Mechanistically, miR-462/miR-731 regulates the *pu.1*-dependent primitive myelopoiesis through targeting *etsrp/scl* pathway. miR-462 and miR-731 also affect *BMP/Smad* signaling in driving primitive myelopoiesis in the ALM (Huang et al. 2019). miR-142-3p is indispensable for the definitive granulosis, whose deficiency leads to hypermaturation of neutrophils. The hypermature neutrophils demonstrated a larger size and declined nucleo-cytoplasmic ratio. The activation of the *IFN-γ* signaling pathway, for instance, the upregulation of *stat1a* and *irf1b*, is responsible for the impaired myelopoiesis caused by miR-142-3p deficiency (Fan et al. 2014). Downstream of transcription factors gata4/5/6, miR-210-5p inhibits primitive myelopoiesis through silencing *foxj1b* and *slc3a2a* (Jia et al. 2019). High expression of miR-129 in myeloid cells tends to favor granulocyte maturation, while lower expression favors monocyte maturation. This is because miR-129 directly suppresses the expression of runx1, which promotes monocyte differentiation yet represses granulocyte differentiation. Interestingly, runx1 in turn affects miR-129 expression at the transcriptional level, forming a feedback regulatory loop (Zhao et al. 2017). miR-223 is up-regulated between 4 and 6 dpf and from 30 dpf to adulthood in the head kidney, a well-described hematopoietic organ in zebrafish considered the equivalent to the bone marrow of mammals, and regulates the seeding of HSCs in the head kidney and definitive hematopoiesis in zebrafish. Due to the evolutional conservative functions between zebrafish and mammalian miR-223, it is speculated that *MEF2C* and *IGF1R* genes are most likely miR-223 targets in zebrafish hematopoiesis, although lacking further validation (Roberto et al. 2015). miR-191 possibly contributes to myelo-monocytic differentiation in zebrafish either, because after hsa-miR-191 was microinjected into the fertilized eggs, the spatiotemporal expression of L-plastin at 24 hpf was specifically up-regulated (Sun et al. 2017).

Telomerase RNA (terc) is able to bind specific DNA sequences, recruits RNA polymerase II, and regulates the expression of myeloid genes (García-Castillo et al. 2021). This function of terc is discovered using a zebrafish *terc* mutant with the CR4-CR5 domain mutation found in patients with dyskeratosis congenita (DC) in vivo and independent on the telomerase catalytic subunit. Telomerase RNA also has a non-canonical function in regulating zebrafish myelopoiesis. Depletion of zebrafish telomerase RNA component (TR) leads to myelopoietic defects without affecting HSC development, in a form independent of telomere length and telomerase activity. This effect also depends on the GCSF and macrophage-stimulating factor (MCSF) mediated regulation of *pu.1* and *gata1* expression (Alcaraz-Pérez et al. 2014).

Recent zebrafish mutant models of myelopoietic disorders

Various zebrafish mutants mimicking human disorders of myelopoiesis have been established (Baeten and de Jong 2018; Xu et al. 2012). In this section, we summarized some of the most recently applied ones. FAM213A has been identified to be associated with a worse prognosis of AML. A zebrafish *fam213a* mutant was used to in vivo validate its role in developmental myelopoiesis, which is a suppressor of p53 (Oh et al. 2020). The Tet methylcytosine dioxygenase 2 (TET2) is generally believed to be involved in the occurrence of hemopathy like myelodysplastic syndrome (MDS) or AML. Recently, Rajan et al. reported that zebrafish larvae with somatic *tet2* loss-of-function mutation demonstrated reduced MPCs, neutrophils, monocytes, and mast cells in definitive myelopoiesis. The emergency myelopoiesis of *tet2* mutants was also impaired, characterized by the leukemia-like excessive production of naïve myeloid cells (Rajan et al. 2022). The *BCR-ABL1* fusion gene is a characteristic of Philadelphia Chromosome (Ph)+CML. Zizioli et al. recently established a new fish line BCR-ABL1pUAS:CFP/hsp70-Gal4, expressing the human *BCR-ABL1*. The larvae carrying the human *BCR-ABL1* fusion gene presented enhanced embryonic myelopoiesis, increased myeloid cells, and suppressed apoptosis. Further study revealed that *BCR-ABL1* induced upregulated myeloid makers like *lmo2*, *pu.1*, and *mpx*, and downregulated erythropoietic markers, affecting hematopoietic cells proliferation in the CHT (Zizioli et al. 2021). Irf8 not only affects the fate determination of myeloid cells in developmental myelopoiesis but also of those in the progression of myeloid neoplasia. Myeloproliferative neoplasm (MPN) is characterized by increased myeloid precursors. *Irf8* mutant zebrafish developed an MPN-like disease with excessive myelopoiesis. One mechanism, irf8 suppressed the *Mertk* pathway in the hematopoietic cells. Thus, irf8 also acts as an evolutionarily conserved neoplastic suppressor (Zhao et al. 2018).

A Noonan syndrome (NS)-like phenotype could be modeled in $Shp2^{D61G}$ mutant zebrafish. $Shp2^{D61G}$ mutant larvae demonstrated defective heart function and juvenile myelomonocytic leukemia (JMML)-like MPN, manifesting excessive myelopoiesis. The $Shp2^{D61G}$ MPCs showed an increased inflammatory response, which may be responsible for this myelopoietic disorder (Solman et al. 2022). The gene *son* encodes an RNA splicing factor and is associated with Down syndrome related hematopoietic disorders. The *son*-deficient zebrafish manifested normal colony-forming capability of HSPCs, while embryonic myelopoiesis and erythropoiesis were both impaired (Belmonte et al. 2021). Ribosomal dysfunction underlies a category of diseases called ribosomopathies in humans, which is characterized by impaired hematopoiesis. Consistently, ribosome biogenesis gene deficiency causes hematopoietic defects in zebrafish (Oyarbide et al. 2019). Several studies revealed that impaired ribosome biogenesis produces excess free ribosomal proteins, which protects p53 from degradation by the E3 ubiquitin ligase MDM2 (Dai and Lu 2004; Dai et al. 2004). There are also p53-independent cell apoptosis and cell proliferation arrest described in ribosomal dysfunction-induced hematopoietic defects. For example, the deficiency of the *LTV1 ribosome biogenesis factor* ($ltv1^{\Delta14/\Delta14}$), a non-ribosomal factor required for the processing of the 40S ribosomal subunit, leads to defective ribosomal biogenesis and impaired definitive hematopoiesis without affecting primitive hematopoiesis. This defect is attributed to decreased proliferation of HSPCs and independent of p53 (Zhang et al. 2021).

Through optimized inter-specific spermatogonial stem cells transplantation (SSCT), Zhang et al. recently successed in the acquirement of sperms carrying edited genome derived from a *gobiocypris rarus* honor from zebrafish recipients (Zhang et al. 2022). It is exciting that if this technology is further improved, it may provide a possibility to construct the zebrafish mutants with specific gene mutations leading to hematopoietic diseases found in any other species. This will enlarge the mutant library of zebrafish with myelopoietic and general hematopoietic disorders on an unprecedented scale.

Limitations and prospects

Even though zebrafish as model organisms for myelopoiesis have been studied for decades, the extremely small size and complicated structure of the zebrafish model still bring great challenges to the in-depth research at the gene level and the cell level. First, the traditional transcriptome sequencing technology is based on multicellular tissue samples, which describes the mean value of signals in a pile of cells and loses the information of cell heterogeneity. It is extremely difficult to separate the tissues and organs of zebrafish embryos and larvae, so the transcriptome sequencing was usually performed on whole-body homogenate of zebrafish embryos and larvae, which further expanded the disadvantage of traditional transcriptome sequencing. The single-cell sequencing technology, which can detect the heterogeneous information of hybrid samples, solves this problem well. In 2021, a full-coverage, single-cell–resolution fate map of zebrafish early foregut endoderm was established through the labeling of endodermal progenitor cells and tracing of its descendant cells (Yang et al. 2021). Through single-cell sequencing, the dynamic state of cell composition and hematopoietic gene expression, as well as the heterogeneity and interaction with niche components of HSPC in the CHT of zebrafish was also depicted (Xia et al. 2021). These studies greatly improve myelopoiesis studies using zebrafish models in both depth and breadth. Second, the progress of microscopic imaging techniques may further facilitate myelopoiesis research using zebrafish models. In 2017, using in vivo long-term two-photon imaging, researchers directly observed the lung microcirculation in a megakaryocyte-specific fluorescent mouse line, identifying the lungs as a huge reservoir for HSPCs (Lefrancais et al. 2017), which has been ignored for nearly a century. If similar imaging technology had been used in similar studies using zebrafish models, this phenomenon might have been discovered much earlier because it is very easy to find the ectopic origin of hematopoietic cells in the long-term observation of transparent zebrafish embryos. Fortunately, a few researchers have noticed this. For example, an ectoderm-derived immune cell population, metaphocytes, has been identified in the epidermis of zebrafish (Lin et al. 2019). By using epidermis- or mesoderm-driven CreERT2 transgenes and long-term confocal imaging, Lin et al. provided convincible evidence for the existence of metaphocytes, which are highly similar to conventional Langerhans cells in transcriptome, morphology, and anatomic location, yet have different origins and functions (Lin et al. 2019). Unlike conventional myeloid cells, endoderm-derived metaphocytes cannot phagocytize apoptotic cells and invading bacteria, but are able to directly obtain soluble antigens in the environment by forming a transepithelial protrusion that passes through keratinocytes on the surface of the skin and transmit the antigens to conventional resident macrophages through apoptosis/phagocytosis axis. Although metaphocytes were initially observed in the epidermis and considered to be derived from ectoderm, in a subsequent study, they were also observed in gills and intestine, and proved to be derived from endoderm (Lin et al. 2020). These studies reveal a general existence of non-hematopoiesis-derived immune cells in zebrafish and challenge the prevalent view that resident immune

cells originate exclusively from the common hematopoiesis process.

There are other defects in zebrafish models limiting their application in myelopoiesis studies. For example, flow cytometry is a widely used technique to isolate and quantitatively characterize myeloid cells in the studies using mouse or rat models. However, the application of flow cytometry depends on commercial fluorescence-labeled antibodies against specific proteins. The biggest problem that restricts the application of flow cytometry in the studies using zebrafish models is the lack of these antibodies against zebrafish. One solution to this problem is to purify specific cells by other methods that do not depend on antibodies. For example, a DNA-staining fluorescent dye, DRAQ5, the primitive erythrocytes could be labeled and then isolated through flow cytometry. Similarly, neutrophils could be distinguished by the combinate of lectin *Phaseolus vulgaris* erythroagglutinin (PHA-E) and DRAQ5 (Konno et al. 2020). Recently, another method of antibody-free flow cytometry isolation of blood cells in zebrafish has been reported, which combines fluorescent fish lines and specific DNA-staining dyes (Konno et al. 2022). This technology is expected to be widely applied. It largely facilitates the application of flow cytometry to the research using zebrafish models and provides another option for the detection of specific gene expression levels, especially when failing to label their mRNAs through routinely used in situ hybridization assays due to the cumbersome steps and possible signal degradation (Wu et al. 2021).

With photopharmacological approach, the first conformationally strained visible light photoswitches (CS-VIPs) have been recently developed as inhibitors of the histone methyltransferase MLL1 (KMT2A) in zebrafish. Through the implantation of CS-VIP 8, in vivo bistable control of hematopoiesis could be fulfilled under visible-light irradiation with unprecedented stability (Albert et al. 2022). Regrettably, in this elegant work, Albert et al. evaluated only erythropoiesis through *o*-dianisidine staining. Promisingly, at least in theory, this technology can also be applied to the optical control of myelopoiesis, if only myeloid cells could be detected through some conventional techniques like WISH, Sudan black staining, and Neutral red staining. In addition, a novel algorithm CellComm emerges and is applied to investigate how the AGM microenvironment dictates HSPC emergence in zebrafish through cell-cell crosstalk (Lummertz da Rocha et al. 2022). As reviewed above, myelopoiesis involves the interaction between HSPC and their niche as well as complex regulatory networks. Hence, CellComm may be applied more specifically in myelopoiesis, if only performed on the data collected from specific position (i.e., the CHT region) in a specific myelopoietic stages (i.e.,

definitive myelopoiesis). It seems that with the development and application of the technologies in bioengineering, bioinformatics, multi-omics, and other disciplines, myelopoiesis research might be even more dependent on zebrafish models in the future.

Because of the relevance and complexity of life activities, genetic manipulations often cause cascade reactions. Therefore, the results of studies using zebrafish models can hardly be directly applied to clinical health care. But it's not just bad news. It is recently reported that a recurrent gain-of-function *ARAF* mutation was found in a 12-year-old boy with advanced anomalous lymphatic disease unresponsive to conventional therapy. Researchers replicated this mutation in zebrafish and successfully validated the functional relevance of this mutation and lymphatic phenotype. An MEK inhibitor was then screened out to rescue the anomalous phenotype in this mutant zebrafish line. Given this known, MEK inhibitor administration improved the patient's symptoms dramatically (Li et al. 2019). In this case, the advantages of the zebrafish model were fully exploited. Zebrafish embryos that had been precisely genetically edited clearly showed how the development of lymphatic vessels was disrupted in real time under the microscope and proved the effectiveness of an MEK inhibitor in vivo. Most excitingly, these experimental results obtained on zebrafish models had achieved magical effects after being applied to the treatment of the patient. In this case, the life-saving zebrafish genetic model provides an invaluable "bench to bedside" experience, and the encore in myelopoietic diseases is full of expectation.

Conclusions

Because of its unique advantages, zebrafish has become a well-recognized model organism for myelopoiesis studies. For decades, the application of zebrafish models has provided new insight into the cognition of myelopoietic physiology and pathophysiology, the correlation between genotype and myelopoietic phenotype, and the exploration of treatment options for myelopoietic diseases. Through precise modeling of specific genetic mutation-caused myelopoietic diseases and high-throughput screening of clinically approved drugs in zebrafish, the customization of treatment schemes for individual patient with myelopoietic disease could be practical, rapid and economical. We envisage that in the next few years zebrafish, as a convenient tool for myelopoiesis research, will more emerge in the translational medicine research on therapeutic drugs for human myelopoietic diseases and actually save more patients' lives.

Abbreviations

AGM: Aorta-gonad-mesonephros; ALM: Anterior lateral mesoderm; AML: Acute myeloid leukemia; C/EBP: CCAAT/enhancer-binding proteins; CHT: Caudal hematopoietic tissue; CML: Chronic myeloid leukemia; CMP: Common myeloid progenitors; Csf1r: Colony-stimulating factor 1 receptor; CS-VIP: Conformationally strained visible light photoswitches; DA: Dorsal aorta; DNMT1: DNA methyltransferase 1; EC: Endothelial cells; EHT: Endothelial hematopoietic transition; EMP: Erythroid-myeloid progenitors; GCSF: Granulocyte colony-stimulating factor; HEC: Hemogenic endothelial cells; hpf: Hours post fertilization; HSC: Hematopoietic stem cells; HSPC: Hematopoietic stem/progenitor cells; IDH: Isocitrate dehydrogenase; JMML: Juvenile myelomonocytic leukemia; MCSF: Macrophage-stimulating factor; MD: Myelodysplastic syndrome; MPC: Myeloid progenitor cells; MPN: Myeloproliferative neoplasm; MSC: Mesenchymal stem cells; PBI: Posterior blood island; Ph: Philadelphia Chromosome; RNF4: Ring finger protein 4; SOCS: Suppressors of cytokine signaling; SSC: Spermatogonial stem cells; STUbL: SUMO-targeted ubiquitin E3 ligase; SUMO: Small ubiquitin-related modifier; TR: Telomerase RNA component; VDA: Ventral wall of the dorsal aorta.

Authors' contributions

YXH: Conceptualization, Software, Writing - Original draft preparation. QJ: Writing - Review & Editing, Project administration, Funding acquisition. The authors read and approved the final manuscript.

Availability of data and materials

Not applicable.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

Author details

[1]Department of Cardiology, Changzheng Hospital, Shanghai 200003, China. [2]CAS Key Laboratory of Tissue Microenvironment and Tumor, Shanghai Institute of Nutrition and Health, Chinese Academy of Sciences, 320 Yue-Yang Road, Shanghai 200031, China.

References

Abidin BM, Hammami A, Stager S, Heinonen KM. Infection-adapted emergency hematopoiesis promotes visceral leishmaniasis. PLoS Pathog. 2017;13(8):e1006422. https://doi.org/10.1371/journal.ppat.1006422.

Albert L, Nagpal J, Steinchen W, Zhang L, Werel L, Djokovic N, et al. Bistable Photoswitch allows in vivo control of hematopoiesis. ACS Cent Sci. 2022;8(1):57–66. https://doi.org/10.1021/acscentsci.1c00434.

Alcaraz-Pérez F, García-Castillo J, García-Moreno D, López-Muñoz A, Anchelin M, Angosto D, et al. A non-canonical function of telomerase RNA in the regulation of developmental myelopoiesis in zebrafish. Nat Commun. 2014;5:3228. https://doi.org/10.1038/ncomms4228.

Baeten JT, de Jong JLO. Genetic models of leukemia in zebrafish. Front Cell Dev Biol. 2018;6:115. https://doi.org/10.3389/fcell.2018.00115.

Banks KM, Lan Y, Evans T. Tet proteins regulate neutrophil granulation in zebrafish through demethylation of socs3b mRNA. Cell Rep. 2021;34(2):108632. https://doi.org/10.1016/j.celrep.2020.108632.

Basu S, Hodgson G, Zhang HH, Katz M, Quilici C, Dunn AR. "Emergency" granulopoiesis in G-CSF–deficient mice in response to Candida albicans infection. Blood. 2000;95(12):3725–33. https://doi.org/10.1182/blood.V95.12.3725.

Belmonte RL, Engbretson IL, Kim JH, Cajias I, Ahn EE, Stachura DL. Son is necessary for proper vertebrate blood development. PLoS One. 2021;16(2):e0247489. https://doi.org/10.1371/journal.pone.0247489.

Bennett CM, Kanki JP, Rhodes J, Liu TX, Paw BH, Kieran MW, et al. Myelopoiesis in the zebrafish, *Danio rerio*. Blood. 2001;98(3):643–51. https://doi.org/10.1182/blood.v98.3.643.

Bertrand JY, Kim AD, Violette EP, Stachura DL, Cisson JL, Traver D. Definitive hematopoiesis initiates through a committed erythromyeloid progenitor in the zebrafish embryo. Development. 2007;134(23):4147–56. https://doi.org/10.1242/dev.012385.

Boyd KE, Xiao YY, Fan K, Poholek A, Copeland NG, Jenkins NA, et al. Sox4 cooperates with Evi1 in AKXD-23 myeloid tumors via transactivation of proviral LTR. Blood. 2006;107(2):733–41. https://doi.org/10.1182/blood-2003-05-1626.

Bresciani E, Carrington B, Wincovitch S, Jones M, Gore AV, Weinstein BM, et al. CBFβ and RUNX1 are required at 2 different steps during the development of hematopoietic stem cells in zebrafish. Blood. 2014;124(1):70–8. https://doi.org/10.1182/blood-2013-10-531988.

Bresciani E, Carrington B, Yu K, Kim EM, Zhen T, Guzman VS, et al. Redundant mechanisms driven independently by RUNX1 and GATA2 for hematopoietic development. Blood Adv. 2021;5(23):4949–62. https://doi.org/10.1182/bloodadvances.2020003969.

Bukrinsky A, Griffin KJ, Zhao Y, Lin S, Banerjee U. Essential role of spi-1-like (spi-1l) in zebrafish myeloid cell differentiation. Blood. 2009;113(9):2038–46. https://doi.org/10.1182/blood-2008-06-162495.

Butko E, Distel M, Pouget C, Weijts B, Kobayashi I, Ng K, et al. Gata2b is a restricted early regulator of hemogenic endothelium in the zebrafish embryo. Development. 2015;142(6):1050–61. https://doi.org/10.1242/dev.119180.

Campbell CA, Fursova O, Cheng X, Snella E, McCune A, Li L, et al. A zebrafish model of granulin deficiency reveals essential roles in myeloid cell differentiation. Blood Adv. 2021;5(3):796–811. https://doi.org/10.1182/bloodadvances.2020003096.

Cao N, Yao ZX. The hemangioblast: from concept to authentication. Anat Rec (Hoboken). 2011;294(4):580–8. https://doi.org/10.1002/ar.21360.

Craig MP, Grajevskaja V, Liao HK, Balciuniene J, Ekker SC, Park JS, et al. Etv2 and fli1b function together as key regulators of vasculogenesis and angiogenesis. Arterioscler Thromb Vasc Biol. 2015;35(4):865–76. https://doi.org/10.1161/ATVBAHA.114.304768.

Dai MS, Lu H. Inhibition of MDM2-mediated p53 ubiquitination and degradation by ribosomal protein L5. J Biol Chem. 2004;279(43):44475–82. https://doi.org/10.1074/jbc.M403722200.

Dai MS, Zeng SX, Jin Y, Sun XX, David L, Lu H. Ribosomal protein L23 activates p53 by inhibiting MDM2 function in response to ribosomal perturbation but not to translation inhibition. Mol Cell Biol. 2004;24(17):7654–68. https://doi.org/10.1128/MCB.24.17.7654-7668.2004.

Dai Y, Zhu L, Huang Z, Zhou M, Jin W, Liu W, et al. Cebpa is essential for the embryonic myeloid progenitor and neutrophil maintenance in zebrafish. J Genet Genomics. 2016;43(10):593–600. https://doi.org/10.1016/j.jgg.2016.09.001.

De Maria R, Zeuner A, Eramo A, Domenichelli C, Bonci D, Grignani F, et al. Negative regulation of erythropoiesis by caspase-mediated cleavage of GATA-1. Nature. 1999;401(6752):489–93. https://doi.org/10.1038/46809.

Detrich HW 3rd, Kieran MW, Chan FY, Barone LM, Yee K, Rundstadler JA, et al. Intraembryonic hematopoietic cell migration during vertebrate development. Proc Natl Acad Sci U S A. 1995;92(23):10713–7. https://doi.org/10.1073/pnas.92.23.10713.

Dong ZW, Ren CG, Xia Y, Su D, Du TT, Fan HB, et al. Pten regulates homeostasis and inflammation-induced migration of myelocytes in zebrafish. J Hematol Oncol. 2014;7:17. https://doi.org/10.1186/1756-8722-7-17.

Dooley KA, Davidson AJ, Zon LI. Zebrafish scl functions independently in hematopoietic and endothelial development. Dev Biol. 2005;277(2):522–36. https://doi.org/10.1016/j.ydbio.2004.09.004.

Dostalova Merkerova M, Krejcik Z, Szikszai K, Kundrat D. Circular RNAs in hematopoiesis with a focus on acute myeloid leukemia and myelodysplastic syndrome. Int J Mol Sci. 2020;21(17):5972. https://doi.org/10.3390/ijms21175972.

Faloon P, Arentson E, Kazarov A, Deng CX, Porcher C, Orkin S, et al. Basic fibroblast growth factor positively regulates hematopoietic development. Development. 2000;127(9):1931–41. https://doi.org/10.1242/dev.127.9.1931.

Fan HB, Liu YJ, Wang L, Du TT, Dong M, Gao L, et al. miR-142-3p acts as an essential modulator of neutrophil development in zebrafish. Blood. 2014;124(8):1320–30. https://doi.org/10.1182/blood-2013-12-545012.

Ferrero G, Miserocchi M, Di Ruggiero E, Wittamer V. A csf1rb mutation uncouples two waves of microglia development in zebrafish. Development. 2021;148(1):dev194241. https://doi.org/10.1242/dev.194241.

Fujii T, Tsunesumi S, Sagara H, Munakata M, Hisaki Y, Sekiya T, et al. Smyd5 plays pivotal roles in both primitive and definitive hematopoiesis during zebrafish embryogenesis. Sci Rep. 2016;6:29157. https://doi.org/10.1038/srep29157.

Gao S, Wang Z, Wang L, Wang H, Yuan H, Liu X, et al. Irf2bp2a regulates terminal granulopoiesis through proteasomal degradation of Gfi1aa in zebrafish. PLoS Genet. 2021;17(8):e1009693. https://doi.org/10.1371/journal.pgen.1009693.

García-Castillo J, Alcaraz-Pérez F, Martínez-Balsalobre E, García-Moreno D, Rossmann MP, Fernández-Lajarín M, et al. Telomerase RNA recruits RNA polymerase II to target gene promoters to enhance myelopoiesis. Proc Natl Acad Sci U S A. 2021;118(32):e2015528118. https://doi.org/10.1073/pnas.2015528118.

Garnett C, Cruz Hernandez D, Vyas P. GATA1 and cooperating mutations in myeloid leukaemia of Down syndrome. IUBMB Life. 2020;72(1):119–30. https://doi.org/10.1002/iub.2197.

Gioacchino E, Koyunlar C, Zink J, de Looper H, de Jong M, Dobrzycki T, et al. Essential role for Gata2 in modulating lineage output from hematopoietic stem cells in zebrafish. Blood Adv. 2021;5(13):2687–700. https://doi.org/10.1182/bloodadvances.2020002993.

Gore AV, Pillay LM, Venero Galanternik M, Weinstein BM. The zebrafish: a fantastic model for hematopoietic development and disease. Wiley Interdiscip Rev Dev Biol. 2018;7(3):e312. https://doi.org/10.1002/wdev.312.

Gutiérrez L, Nikolic T, van Dijk TB, Hammad H, Vos N, Willart M, et al. Gata1 regulates dendritic-cell development and survival. Blood. 2007;110(6):1933–41. https://doi.org/10.1182/blood-2006-09-048322.

Hall CJ, Flores MV, Oehlers SH, Sanderson LE, Lam EY, Crosier KE, et al. Infection-responsive expansion of the hematopoietic stem and progenitor cell compartment in zebrafish is dependent upon inducible nitric oxide. Cell Stem Cell. 2012;10(2):198–209. https://doi.org/10.1016/j.stem.2012.01.007.

Hason M, Mikulasova T, Machonova O, Pombinho A, van Ham TJ, Irion U, et al. M-CSFR/CSF1R signaling regulates myeloid fates in zebrafish via distinct action of its receptors and ligands. Blood Adv. 2022;6(5):1474–88. https://doi.org/10.1182/bloodadvances.2021005459.

Hirai H, Yokota A, Tamura A, Sato A, Maekawa T. Non-steady-state hematopoiesis regulated by the C/EBPβ transcription factor. Cancer Sci. 2015;106(7):797–802. https://doi.org/10.1111/cas.12690.

Hockings C, Deaner V, Hoade Y, Dace P, Lubin A, Payne E. A zebrafish model of cooperating C and N terminal CEBPA mutations reveals defects in early Myelopoeisis and HSPCs leading to Leukaemogenesis. Blood. 2018;132:1343. https://doi.org/10.1182/blood-2018-99-119336.

Hockings C, Rowell J, Pietka G, Contento G, Pena O, Payne EM. A zebrafish model to study co-operating mutations in CEBPA-mutated AML. Blood. 2016;128(22):1552. https://doi.org/10.1182/blood.V128.22.1552.1552.

Holtschke T, Löhler J, Kanno Y, Fehr T, Giese N, Rosenbauer F, et al. Immunodeficiency and chronic myelogenous leukemia-like syndrome in mice with a targeted mutation of the ICSBP gene. Cell. 1996;87(2):307–17. https://doi.org/10.1016/s0092-8674(00)81348-3.

Hou HA, Lu JW, Lin TY, Tsai CH, Chou WC, Lin CC, et al. Clinico-biological significance of suppressor of cytokine signaling 1 expression in acute myeloid leukemia. Blood Cancer J. 2017;7(7):e588. https://doi.org/10.1038/bcj.2017.67.

Hou Y, Sheng Z, Mao X, Li C, Chen J, Zhang J, et al. Systemic inoculation of Escherichia coli causes emergency myelopoiesis in zebrafish larval caudal hematopoietic tissue. Sci Rep. 2016;6:36853. https://doi.org/10.1038/srep36853.

Huang CX, Huang Y, Duan XK, Zhang M, Tu JP, Liu JX, et al. Zebrafish miR-462-731 regulates hematopoietic specification and pu.1-dependent primitive myelopoiesis. Cell Death Differ. 2019;26(8):1531–44. https://doi.org/10.1038/s41418-018-0234-0.

Huang Z, Chen K, Chi Y, Jin H, Li L, Zhang W, et al. Runx1 regulates zebrafish neutrophil maturation via synergistic interaction with c-Myb. J Biol Chem. 2021;296:100272. https://doi.org/10.1016/j.jbc.2021.100272.

Jia W, Liang D, Li N, Liu M, Dong Z, Li J, et al. Zebrafish microRNA miR-210-5p inhibits primitive myelopoiesis by silencing foxj1b and slc3a2a mRNAs downstream of gata4/5/6 transcription factor genes. J Biol Chem. 2019;294(8):2732–43. https://doi.org/10.1074/jbc.RA118.005079.

Jin H, Huang Z, Chi Y, Wu M, Zhou R, Zhao L, et al. c-Myb acts in parallel and cooperatively with Cebp1 to regulate neutrophil maturation in zebrafish. Blood. 2016;128(3):415–26. https://doi.org/10.1182/blood-2015-12-686147.

Kafina MD, Paw BH. Using the zebrafish as an approach to examine the mechanisms of vertebrate erythropoiesis. Methods Mol Biol. 2018;1698:11–36. https://doi.org/10.1007/978-1-4939-7428-3_2.

Kennedy M, D'Souza SL, Lynch-Kattman M, Schwantz S, Keller G. Development of the hemangioblast defines the onset of hematopoiesis in human ES cell differentiation cultures. Blood. 2007;109(7):2679–87. https://doi.org/10.1182/blood-2006-09-047704.

Ketharnathan S, Labudina A, Horsfield JA. Cohesin components Stag1 and Stag2 differentially influence Haematopoietic mesoderm development in zebrafish embryos. Front Cell Dev Biol. 2020;8:617545. https://doi.org/10.3389/fcell.2020.617545.

Kim M, Civin CI, Kingsbury TJ. MicroRNAs as regulators and effectors of hematopoietic transcription factors. Wiley Interdiscip Rev RNA. 2019;10(5):e1537. https://doi.org/10.1002/wrna.1537.

Kissa K, Herbomel P. Blood stem cells emerge from aortic endothelium by a novel type of cell transition. Nature. 2010;464(7285):112–5. https://doi.org/10.1038/nature08761.

Konno K, Kobayashi-Sun J, Arai F, Kobayashi I, Sugiyama D. Hematopoietic cell isolation by antibody-free flow cytometry in the zebrafish embryo. Methods Mol Biol. 2022;2520:171–80. https://doi.org/10.1007/7651_2021_459.

Konno K, Kulkeaw K, Sasada M, Nii T, Kaneyuki A, Ishitani T, et al. A novel method to purify neutrophils enables functional analysis of zebrafish hematopoiesis. Genes Cells. 2020;25(12):770–81. https://doi.org/10.1111/gtc.12810.

Kwon HB, Mackie DI, Bonnavion R, Mercier AL, Helker CSM, Son T, et al. The orphan G-protein coupled receptor 182 is a negative regulator of definitive hematopoiesis through leukotriene B4 signaling. ACS Pharmacol Transl Sci. 2020;3(4):676–89. https://doi.org/10.1021/acsptsci.0c00020.

Le Guyader D, Redd MJ, Colucci-Guyon E, Murayama E, Kissa K, Briolat V, et al. Origins and unconventional behavior of neutrophils in developing zebrafish. Blood. 2008;111(1):132–41. https://doi.org/10.1182/blood-2007-06-095398.

Lefrancais E, Ortiz-Munoz G, Caudrillier A, Mallavia B, Liu F, Sayah DM, et al. The lung is a site of platelet biogenesis and a reservoir for haematopoietic progenitors. Nature. 2017;544(7648):105–9. https://doi.org/10.1038/nature21706.

Li D, March ME, Gutierrez-Uzquiza A, Kao C, Seiler C, Pinto E, et al. ARAF recurrent mutation causes central conducting lymphatic anomaly treatable with a MEK inhibitor. Nat Med. 2019;25(7):1116–22. https://doi.org/10.1038/s41591-019-0479-2.

Li FF, Liang YL, Han XS, Guan YN, Chen J, Wu P, et al. ADP receptor P2y12 prevents excessive primitive hematopoiesis in zebrafish by inhibiting Gata1. Acta Pharmacol Sin. 2021;42(3):414–21. https://doi.org/10.1038/s41401-020-0431-5.

Lian J, Chen J, Wang K, Zhao L, Meng P, Yang L, et al. Alas1 is essential for neutrophil maturation in zebrafish. Haematologica. 2018;103(11):1785–95. https://doi.org/10.3324/haematol.2018.194316.

Lin X, Zhou Q, Lin G, Zhao C, Wen Z. Endoderm-derived myeloid-like Metaphocytes in zebrafish gill mediate soluble antigen-induced immunity. Cell Rep. 2020;33(1):108227. https://doi.org/10.1016/j.celrep.2020.108227.

Lin X, Zhou Q, Zhao C, Lin G, Xu J, Wen Z. An ectoderm-derived myeloid-like cell population functions as antigen transporters for Langerhans cells in zebrafish epidermis. Dev Cell. 2019;49(4):605–17.e5. https://doi.org/10.1016/j.devcel.2019.03.028.

Liu W, Di Q, Li K, Li J, Ma N, Huang Z, et al. The synergistic role of Pu.1 and Fms in zebrafish osteoclast-reducing osteopetrosis and possible therapeutic strategies. J Genet Genomics. 2020;47(9):535–46. https://doi.org/10.1016/j.jgg.2020.09.002.

Liu W, Wu M, Huang Z, Lian J, Chen J, Wang T, et al. C-myb hyperactivity leads to myeloid and lymphoid malignancies in zebrafish. Leukemia. 2017;31(1):222–33. https://doi.org/10.1038/leu.2016.170.

Lu JW, Hsieh MS, Hou HA, Chen CY, Tien HF, Lin LI. Overexpression of SOX4 correlates with poor prognosis of acute myeloid leukemia and is leukemogenic in zebrafish. Blood Cancer J. 2017;7(8):e593. https://doi.org/10.1038/bcj.2017.74.

Lummertz da Rocha E, Kubaczka C, Sugden WW, Najia MA, Jing R, Markel A, et al. CellComm infers cellular crosstalk that drives haematopoietic stem and progenitor cell development. Nat Cell Biol. 2022;24(4):579–89. https://doi.org/10.1038/s41556-022-00884-1.

Maglitto A, Mariani SA, de Pater E, Rodriguez-Seoane C, Vink CS, Piao X, et al. Unexpected redundancy of Gpr56 and Gpr97 during hematopoietic cell development and differentiation. Blood Adv. 2021;5(3):829–42. https://doi.org/10.1182/bloodadvances.2020003693.

Mallampati S, Sun B, Lu Y, Ma H, Gong Y, Wang D, et al. Integrated genetic approaches identify the molecular mechanisms of Sox4 in early B-cell development: intricate roles for RAG1/2 and CK1epsilon. Blood. 2014;123(26):4064–76. https://doi.org/10.1182/blood-2013-12-543801.

Meier AB, Basheer F, Sertori R, Laird M, Liongue C, Ward AC. Granulocyte Colony-stimulating factor mediated regulation of early myeloid cells in zebrafish. Front Biosci (Landmark Ed). 2022;27(4):110. https://doi.org/10.31083/j.fbl2704110.

Migliaccio AR, Rana RA, Sanchez M, Lorenzini R, Centurione L, Bianchi L, et al. GATA-1 as a regulator of mast cell differentiation revealed by the phenotype of the GATA-1low mouse mutant. J Exp Med. 2003;197(3):281–96. https://doi.org/10.1084/jem.20021149.

Morrison SJ, Kimble J. Asymmetric and symmetric stem-cell divisions in development and cancer. Nature. 2006;441(7097):1068–74. https://doi.org/10.1038/nature04956.

Morrison SJ, Spradling AC. Stem cells and niches: mechanisms that promote stem cell maintenance throughout life. Cell. 2008;132(4):598–611. https://doi.org/10.1016/j.cell.2008.01.038.

Murayama E, Kissa K, Zapata A, Mordelet E, Briolat V, Lin HF, et al. Tracing hematopoietic precursor migration to successive hematopoietic organs during zebrafish development. Immunity. 2006;25(6):963–75. https://doi.org/10.1016/j.immuni.2006.10.015.

Oh CK, Ha M, Han ME, Heo HJ, Myung K, Lee Y, et al. FAM213A is linked to prognostic significance in acute myeloid leukemia through regulation of oxidative stress and myelopoiesis. Hematol Oncol. 2020;38(3):381–9. https://doi.org/10.1002/hon.2728.

Oyarbide U, Topczewski J, Corey SJ. Peering through zebrafish to understand inherited bone marrow failure syndromes. Haematologica. 2019;104(1):13–24. https://doi.org/10.3324/haematol.2018.196105.

Pak B, Schmitt CE, Oh S, Kim JD, Choi W, Han O, et al. Pax9 is essential for granulopoiesis but dispensable for erythropoiesis in zebrafish. Biochem Biophys Res Commun. 2021;534:359–66. https://doi.org/10.1016/j.bbrc.2020.11.077.

Patterson LJ, Gering M, Eckfeldt CE, Green AR, Verfaillie CM, Ekker SC, et al. The transcription factors Scl and Lmo2 act together during development of the hemangioblast in zebrafish. Blood. 2007;109(6):2389–98. https://doi.org/10.1182/blood-2006-02-003087.

Peña OA, Lubin A, Rowell J, Hoade Y, Khokhar N, Lemmik H, et al. Differential requirement of Gata2a and Gata2b for primitive and definitive myeloid development in zebrafish. Front Cell Dev Biol. 2021;9:708113. https://doi.org/10.3389/fcell.2021.708113.

Qin Y, Zhang C. The regulatory role of IFN-γ on the proliferation and differentiation of hematopoietic stem and progenitor cells. Stem Cell Rev Rep. 2017;13(6):705–12. https://doi.org/10.1007/s12015-017-9761-1.

Qiu Y, Xu M, Huang S. Long noncoding RNAs: emerging regulators of normal and malignant hematopoiesis. Blood. 2021;138(23):2327–36. https://doi.org/10.1182/blood.2021011992.

Rajan V, Collett K, Woodside R, Prykhozhij SV, Moksa M, Carles A, et al. Stress hematopoiesis induces a proliferative advantage in TET2 deficiency. Leukemia. 2022;36(3):809–20. https://doi.org/10.1038/s41375-021-01427-7.

Roberto VP, Tiago DM, Gautvik K, Cancela ML. Evidence for the conservation of miR-223 in zebrafish (Danio rerio): implications for function. Gene. 2015;566(1):54–62. https://doi.org/10.1016/j.gene.2015.04.022.

Robertson AL, Avagyan S, Gansner JM, Zon LI. Understanding the regulation of vertebrate hematopoiesis and blood disorders - big lessons from a small fish. FEBS Lett. 2016;590(22):4016–33. https://doi.org/10.1002/1873-3468.12415.

Sandoval S, Kraus C, Cho EC, Cho M, Bies J, Manara E, et al. Sox4 cooperates with CREB in myeloid transformation. Blood. 2012;120(1):155–65. https://doi.org/10.1182/blood-2011-05-357418.

Schim van der Loeff I, Sprenkeler EGG, Tool ATJ, Abinun M, Grainger A, Engelhardt KR, et al. Defective neutrophil development and specific granule deficiency caused by a homozygous splice-site mutation in SMARCD2. J Allergy Clin Immunol. 2021;147(6):2381–5.e2. https://doi.org/10.1016/j.jaci.2020.11.025.

Shi X, He BL, Ma AC, Guo Y, Chi Y, Man CH, et al. Functions of idh1 and its mutation in the regulation of developmental hematopoiesis in zebrafish. Blood. 2015;125(19):2974–84. https://doi.org/10.1182/blood-2014-09-601187.

Shiau CE, Kaufman Z, Meireles AM, Talbot WS. Differential requirement for irf8 in formation of embryonic and adult macrophages in zebrafish. PLoS One. 2015;10(1):e0117513. https://doi.org/10.1371/journal.pone.0117513.

Solman M, Blokzijl-Franke S, Piques F, Yan C, Yang Q, Strullu M, et al. Inflammatory response in hematopoietic stem and progenitor cells triggered by activating SHP2 mutations evokes blood defects. Elife. 2022;11:e73040. https://doi.org/10.7554/eLife.73040.

Stachura DL, Svoboda O, Campbell CA, Espín-Palazón R, Lau RP, Zon LI, et al. The zebrafish granulocyte colony-stimulating factors (Gcsfs): 2 paralogous cytokines and their roles in hematopoietic development and maintenance. Blood. 2013;122(24):3918–28. https://doi.org/10.1182/blood-2012-12-475392.

Sumanas S, Lin S. Ets1-related protein is a key regulator of vasculogenesis in zebrafish. PLoS Biol. 2006;4(1):e10. https://doi.org/10.1371/journal.pbio.0040010.

Sun CG, Chen M, Ruan Z, Han RQ, Wang WT, Wang F, et al. Study on Myelopoietic functions of miR-191 by using zebrafish model. Zhongguo Shi Yan Xue Ye Xue Za Zhi. 2017;25(4):1172–7. https://doi.org/10.7534/j.issn.1009-2137.2017.04.037.

Sun J, Liu W, Li L, Chen J, Wu M, Zhang Y, et al. Suppression of Pu.1 function results in expanded myelopoiesis in zebrafish. Leukemia. 2013;27(9):1913–7. https://doi.org/10.1038/leu.2013.67.

Tamplin OJ, Durand EM, Carr LA, Childs SJ, Hagedorn EJ, Li P, et al. Hematopoietic stem cell arrival triggers dynamic remodeling of the perivascular niche. Cell. 2015;160(1-2):241–52. https://doi.org/10.1016/j.cell.2014.12.032.

Tamura T, Thotakura P, Tanaka TS, Ko MSH, Ozato K. Identification of target genes and a unique cis element regulated by IRF-8 in developing macrophages. Blood. 2005;106(6):1938–47. https://doi.org/10.1182/blood-2005-01-0080.

Thompson MA, Ransom DG, Pratt SJ, MacLennan H, Kieran MW, Detrich HW 3rd, et al. The cloche and spadetail genes differentially affect hematopoiesis and vasculogenesis. Dev Biol. 1998;197(2):248–69. https://doi.org/10.1006/dbio.1998.8887.

Tregnago C, Manara E, Zampini M, Bisio V, Borga C, Bresolin S, et al. CREB engages C/EBPδ to initiate leukemogenesis. Leukemia. 2016;30(9):1887–96. https://doi.org/10.1038/leu.2016.98.

Tyrkalska SD, Pérez-Oliva AB, Rodríguez-Ruiz L, Martínez-Morcillo FJ, Alcaraz-Pérez F, Martínez-Navarro FJ, et al. Inflammasome regulates hematopoiesis through cleavage of the master erythroid transcription factor GATA1. Immunity. 2019;51(1):50–63.e5. https://doi.org/10.1016/j.immuni.2019.05.005.

Ueda Y, Cain DW, Kuraoka M, Kondo M, Kelsoe G. IL-1R type I-dependent hemopoietic stem cell proliferation is necessary for inflammatory granulopoiesis and reactive neutrophilia. J Immunol. 2009;182(10):6477–84. https://doi.org/10.4049/jimmunol.0803961.

Ulloa BA, Habbsa SS, Potts KS, Lewis A, McKinstry M, Payne SG, et al. Definitive hematopoietic stem cells minimally contribute to embryonic hematopoiesis. Cell Rep. 2021;36(11):109703. https://doi.org/10.1016/j.celrep.2021.109703.

Vogeli KM, Jin SW, Martin GR, Stainier DY. A common progenitor for haematopoietic and endothelial lineages in the zebrafish gastrula. Nature. 2006;443(7109):337–9. https://doi.org/10.1038/nature05045.

Wang L, Gao S, Wang H, Xue C, Liu X, Yuan H, et al. Interferon regulatory factor 2 binding protein 2b regulates neutrophil versus macrophage fate during zebrafish definitive myelopoiesis. Haematologica. 2020;105(2):325–37. https://doi.org/10.3324/haematol.2019.217596.

Wang L, Liu X, Wang H, Yuan H, Chen S, Chen Z, et al. RNF4 regulates zebrafish granulopoiesis through the DNMT1-C/EBPα axis. FASEB J. 2018a;32(9):4930–40. https://doi.org/10.1096/fj.201701450RR.

Wang L, Wang X, Wang L, Yousaf M, Li J, Zuo M, et al. Identification of a new adtrp1-tfpi regulatory axis for the specification of primitive myelopoiesis and definitive hematopoiesis. FASEB J. 2018b;32(1):183–94. https://doi.org/10.1096/fj.201700166RR.

Wei CY, Wang HP, Zhu ZY, Sun YH. Transcriptional factors smad1 and smad9 act redundantly to mediate zebrafish ventral specification downstream of smad5. J Biol Chem. 2014;289(10):6604–18. https://doi.org/10.1074/jbc.M114.549758.

Wei Z, Li C, Zhang Y, Lin C, Zhang Y, Shu L, et al. Macrophage-derived IL-1β regulates emergency Myelopoiesis via the NF-κB and C/ebpβ in zebrafish. J Immunol. 2020;205(10):2694–706. https://doi.org/10.4049/jimmunol.2000473.

Witzel M, Petersheim D, Fan Y, Bahrami E, Racek T, Rohlfs M, et al. Chromatin-remodeling factor SMARCD2 regulates transcriptional networks controlling differentiation of neutrophil granulocytes. Nat Genet. 2017;49(5):742–52. https://doi.org/10.1038/ng.3833.

Wu JM, Galibois AA, Seebocus N, Apu A, Hnatovska M, Wilson A, et al. HER2 fluorescent in situ hybridization signal degradation: a 10-year retrospective study. Breast Cancer Res Treat. 2021;186(1):99–105. https://doi.org/10.1007/s10549-020-06048-9.

Xia J, Kang Z, Xue Y, Ding Y, Gao S, Zhang 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(14):e2015748118. https://doi.org/10.1073/pnas.2015748118.

Xu J, Du L, Wen Z. Myelopoiesis during zebrafish early development. J Genet Genomics. 2012;39(9):435–42. https://doi.org/10.1016/j.jgg.2012.06.005.

Xu J, Zhu L, He S, Wu Y, Jin W, Yu T, et al. Temporal-spatial resolution fate mapping reveals distinct origins for embryonic and adult microglia in zebrafish. Dev Cell. 2015;34(6):632–41. https://doi.org/10.1016/j.devcel.2015.08.018.

Yang Y, Wang H, He J, Shi W, Jiang Z, Gao L, et al. A single-cell-resolution fate map of endoderm reveals demarcation of pancreatic progenitors by cell cycle. Proc Natl Acad Sci U S A. 2021;118(25):e2025793118. https://doi.org/10.1073/pnas.2025793118.

Yu C, Cantor AB, Yang H, Browne C, Wells RA, Fujiwara Y, et al. Targeted deletion of a high-affinity GATA-binding site in the GATA-1 promoter leads to selective loss of the eosinophil lineage in vivo. J Exp Med. 2002;195(11):1387–95. https://doi.org/10.1084/jem.20020656.

Yu SH, Zhu KY, Zhang F, Wang J, Yuan H, Chen Y, et al. The histone demethylase Jmjd3 regulates zebrafish myeloid development by promoting spi1 expression. Biochim Biophys Acta Gene Regul Mech. 2018;1861(2):106–16. https://doi.org/10.1016/j.bbagrm.2017.12.009.

Yu T, Guo W, Tian Y, Xu J, Chen J, Li L, et al. Distinct regulatory networks control the development of macrophages of different origins in zebrafish. Blood. 2017;129(4):509–19. https://doi.org/10.1182/blood-2016-07-727651.

Zang L, Torraca V, Shimada Y, Nishimura N. Editorial: zebrafish models for human disease studies. Front Cell Dev Biol. 2022;10:861941. https://doi.org/10.3389/fcell.2022.861941.

Zhang C, Huang R, Ma X, Chen J, Han X, Li L, et al. The ribosome biogenesis factor Ltv1 is essential for digestive organ development and definitive hematopoiesis in zebrafish. Front Cell Dev Biol. 2021;9:704730. https://doi.org/10.3389/fcell.2021.704730.

Zhang F, Hao Y, Li X, Li Y, Ye D, Zhang R, et al. Surrogate production of genome-edited sperm from a different subfamily by spermatogonial stem cell transplantation. Sci China Life Sci. 2022;65(5):969–87. https://doi.org/10.1007/s11427-021-1989-9.

Zhang H, Ye M, Welner RS, Tenen DG. Sox4 is required for the formation and maintenance of multipotent progenitors. Blood. 2014;124(21):1577. https://doi.org/10.1182/blood.V124.21.1577.1577.

Zhang Y, Jin H, Li L, Qin FX, Wen Z. cMyb regulates hematopoietic stem/progenitor cell mobilization during zebrafish hematopoiesis. Blood. 2011;118(15):4093–101. https://doi.org/10.1182/blood-2011-03-342501.

Zhao F, Shi Y, Huang Y, Zhan Y, Zhou L, Li Y, et al. Irf8 regulates the progression of myeloproliferative neoplasm-like syndrome via Mertk signaling in zebrafish. Leukemia. 2018;32(1):149–58. https://doi.org/10.1038/leu.2017.189.

Zhao H, Wang X, Yi P, Si Y, Tan P, He J, et al. KSRP specifies monocytic and granulocytic differentiation through regulating miR-129 biogenesis and RUNX1 expression. Nat Commun. 2017;8(1):1428. https://doi.org/10.1038/s41467-017-01425-3.

Zizioli D, Bernardi S, Varinelli M, Farina M, Mignani L, Bosio K, et al. Development of BCR-ABL1 transgenic zebrafish model reproducing chronic myeloid leukemia (CML) like-disease and providing a new insight into CML mechanisms. Cells. 2021;10(2):445. https://doi.org/10.3390/cells10020445.