Fish to Learn: Insights into Blood Development and Blood Disorders from Zebrafish Hematopoiesis

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Since its introduction in early 1980s, the zebrafish (*Danio rerio*) has become an invaluable vertebrate animal model system to study many human disorders in almost all systems, from hepatic and brain pathology, to autoimmune and psychiatric disorders. Hematopoiesis between zebrafish and mammals is highly conserved, making the zebrafish an attractive model to study hematopoietic development and blood disorders. Unique attributes of the zebrafish include the ability to perform large-scale genetic and chemical screens *in vivo*, study development at the cellular level, and use transgenic fish to dissect mechanisms of disease or drug effects. This review summarizes major discoveries that helped define molecular control of hematopoiesis in vertebrates and specific contributions from studies in zebrafish.

INTRODUCTION

SINCE ITS INTRODUCTION as a vertebrate model system in early 1980s, the zebrafish (*Danio rerio*) has emerged as an important and unique genetic system to study the hematopoietic system, in both normal development and hematopoietic disorders. Among the advantages of the zebrafish are external fertilization, allowing direct visualization of developmental processes from the one-cell embryonic stage, ability to generate hundreds of embryos at a time from one mating, making it a powerful system for forward genetic and chemical screens, and fast development in the order of a few days in contrast to other vertebrate models.

The hematopoietic system in particular has been extensively studied in the zebrafish since early 1990s. Even though the sites of hematopoietic development are different in the zebrafish compared with mammals, the genetic program regulating hematopoiesis is largely conserved, permitting translation of discoveries to mammals. Forward genetic screens to date have identified several genes important in hematopoietic development that not only described novel functions of these genes in hematopoiesis, but also elucidated previously uncharacterized human hematopoietic disorders associated with mutations in these genes.[1] Chemical screens in the zebrafish have been promising in identifying small molecules and biologically active compounds that affect various aspects of hematopoiesis, and show significant effect in mammalian models and, in some unique cases, in human patients. With the advent of various new techniques in genome editing and clonal lineage tracking, and the growing wealth of genetic information in human hematopoietic diseases, it has been tremendously exciting to continue the studies of hematopoiesis and hematopoietic disorders using the zebrafish. Here, we will give a general overview of vertebrate hematopoiesis as modeled in the zebrafish and review the most recent advances in the field.

OVERVIEW OF ZEBRAFISH HEMATOPOIESIS

Gastrulation in the zebrafish, like in all vertebrates, gives rise to three germ layers—ectoderm, mesoderm, and endoderm—that specify the various tissues in the organism. Mesoderm defines a dorsal fate in a group of cells that become the somites and the notochord, and a ventral fate in another group of cells, which eventually become

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blood, the vasculature, and pronephros. The hematopoietic program is characterized by two waves of development—a primary wave, which is transient and has the primary role of supporting early developmental stages, and a secondary wave, which gives rise to the definitive hematopoietic system that maintains it for the lifetime of the organism.

The primitive wave gives rise to primitive erythroid and myeloid cells.[2] The role of the primitive wave has been suggested to be largely supportive of the early developmental stages. The primitive erythroid cells stem from the posterior part of the embryo, the posterior lateral mesoderm, and more specifically from an area called intermediate cell mass (ICM).[3] These cells provide tissue oxygenation as the embryo grows.[4] Primitive macrophages arise from the anterior portion of the embryo, the anterior lateral mesoderm. The role of the primitive macrophages is still an area of active research. Recent studies have suggested an important role of primitive macrophages in facilitating establishment of definitive hematopoietic stem and progenitor cells (HSPCs) in their early niche at the aorto-gonadal-mesonephros.[5] Around 24 hr postfertilization (hpf), the primitive blood cells begin to circulate throughout the embryo.

The definitive hematopoietic wave is classically accepted as the developmental step that gives rise to the hematopoietic stem cells (HSCs) of the organism that will persist throughout lifetime. HSCs are capable of self-renewing and producing all blood lineages *via* differentiation, with tightly orchestrated gene control over these two processes. The birthplace of the definitive HSC beginning around 30 hpf is in the ventral wall of the dorsal aorta, a region termed aorta-gonad-mesonephros (AGM).[6,7] By 36 hpf these cells start migrating toward the posterior region of the zebrafish tail into a region termed caudal hematopoietic tissue (CHT).[8,9] Some HSCs migrate directly into the thymus, where lymphopoiesis starts around 3 days postfertilization (dpf). Most of the HSCs, however, go through the CHT and continuously circulate in the blood, until about 4 dpf when they seed the kidney marrow. The kidney marrow is the equivalent of the mammalian bone marrow, the final residence of HSCs and lifetime hematopoiesis.

Despite different anatomical sites of origin and migratory pattern of HSCs, zebrafish and mammalian hematopoiesis share genetic regulation of HSC development and lineages specification. This makes discoveries in the zebrafish blood development relevant to mammalian and specifically human hematopoiesis. Over the past years, several examples of such findings have proven this notion. Importantly, not only have the genes identified in the mammalian models been studied in zebrafish, but also newly discovered pathways from forward genetic screen in zebrafish or new biologically active compounds controlling zebrafish HSCs have been validated in mammals and in some cases in humans. This makes the zebrafish a valuable model system to study the hematopoietic system using forward genetics, chemical screens, live imaging of hematopoietic development at a single-cell level, and modeling hematopoietic disorders and malignancies using transgenic zebrafish.

FORWARD GENETIC SCREENS IN ZEBRAFISH

The ease of genetic manipulation of zebrafish embryos is comparable to no other vertebrate system. The zebrafish are amenable to large-scale genetic screens at a low cost and space requirement.[10] The *ex vivo* development of the embryos allows for microinjection of RNA or DNA, to knockdown or overexpress specific genes to assess their molecular function in an effective fashion. More recently, newer technologies, such as TALENs[11] and CRISPR-Cas9 system,[12] have been adapted to zebrafish, expanding the possibilities of site-specific genetic targeting.

There have been several large-scale forward genetic screens performed in the zebrafish using the chemical mutagen ENU (*N*-ethyl-*N*-nitrosourea) or retroviral mutagenesis.[13–17] Two large ENU screens, performed about two decades ago, yielded a number of interesting mutants in the hematopoietic system, which exhibited hematopoietic defects such as impaired HSC differentiation, or abnormalities of specific lineages. These mutants not only helped decipher specific genetic pathways controlling the hematopoietic development, but also defined human diseases that did not yet have an identified genetic mutation in human patients. It is important to note that an additional advantage of the zebrafish embryos is that they can survive without red blood cells, by passive oxygen diffusion, during the initial stages of development, for up to one week. This allows identification of genetic lesions critical for the early stages of hematopoietic development, both in primitive and early definitive hematopoiesis, that would otherwise be impossible in a mammalian system as most would be embryonic lethal in mice.

Large numbers of the genetic defects identified in the above-mentioned screens were in transcription factors. The *cloche* mutant has defects in both hematopoietic and endothelial differentiation,

suggesting a defect in the hemangioblast specification that gives rise to both tissue types.[18] The *spadetail* mutant carries a mutation in the *tbx16* gene resulting in a defect in mesoderm-derived tissues, including the blood, and shown to be important for hemangioblast regulation. This genetic mutation disrupts trunk somite formation and leads to abnormal ICM and AGM blood precursors, and specifically affects the posterior hematopoietic progenitor formation that gives rise to primitive myeloid cells.[6] With respect to lineage-specific mutants, the *vlad tepes* mutant shows no erythroid cells and is characterized by a nonsense point mutation in the C-terminus of gata1, underlying the importance of this transcription factor in erythropoiesis. The mechanism of this mutation leading to defective erythroid differentiation is attributable to inability of the mutated gata1 to bind to the promoter region of its target genes to initiate the erythroid program.[19]

One of the most impressive success stories revealing a novel mechanism of control of erythropoiesis in vertebrates comes from studies of the *moonshine* mutant. This mutant is peculiar as it had specification of both primitive and definitive erythroid progenitors, as evidenced by early expression of *gata1* and *scl* at 5 somite stage (around 11 hpf); however, these cells undergo cell death and are unable to terminally differentiate.[20] Expression of *gata1, scl,* and *gata2* is absent by 22 hpf. Positional cloning in zebrafish identified the underlying mutation in an ortholog of transcription intermediary factor 1γ (TIF1γ). An innovative approach in a *tif1*$\gamma^{-/-}$ fish line using a genetic suppressor screen elucidated the mechanism of action of tif1γ in blood formation.[21] The screen identified that a mutation in the *cdc73* gene, encoding for a component of Pol-II-associated factor (PAF) complex, as well as mutations in genes encoding for other components of the PAF complex and another complex called DSIF (1-β-D-ribofuranosylbenzimidazole sensitivity-induced factor), restored erythropoiesis in the *moonshine* mutant. Both these complexes are known to stall the RNA polymerase II (Pol II). It appears that tif1γ recruits positive elongation factors to erythroid genes *via* its interaction with scl transcription complex, and releases the stalled Pol II, allowing transcription of these genes. Studies in mammals validated this mechanism first described in the zebrafish.[22,23]

Another curious mutant described from the insertional mutagenesis screen is the *bloodless* mutant, which is missing erythroid cells from the primitive hematopoietic wave, and survives solely on diffused oxygen. No erythroid cells are present in this mutant until about 5 dpf, at which point the blood recovers likely from the definitive hematopoietic wave and the fish survive to adulthood.[24] The gene underlying this mutant is yet to be discovered.

The forward genetic screens also identified some genes later to be shown to be important in human disease. One such example is the hypochromic anemic mutant *weissherbst*, which is caused by a mutation in a previously unknown iron transporter, *ferroportin 1*,[25] later identified in patients with type IV autosomal dominant form of hemochromatosis.[26,27] Another example came from the mutant *shiraz* with hypochromic microcytic anemia, characterized by glutaredoxin 5 (grx5) deficiency required for iron–sulphur cluster formation important for early steps in heme biosynthesis involving the enzyme delta-aminolevulinate synthase 2 (alas2).[28] A recessive mutation in *GRX5* was later identified in a human patient with a similar clinical phenotype.[29]

In addition to the early genetic screens discussed above, various other screens have been performed in zebrafish focusing on specific hematopoietic lineages and hematopoietic phenotypes.[30–32] New tools in the field of forward genetics have emerged in the past decade using RNA interference (RNAi) for gene knockdown. Most recently, sequence-specific RNA-guided endonuclease Cas9 from the bacterial immune system CRISPR presented an effective and efficient system to target the eukaryotic genome. We recently published a porphyria model using a tissue-specific CRISPR technique.[33] CRISPR-Cas9-based screens will certainly be incorporated in forward genetic studies in zebrafish in the near future.

CHEMICAL SCREENS IN ZEBRAFISH

Zebrafish have *ex vivo* ontogeny, which is transparent for the first several days of development, and can be easily soaked in chemical-containing solutions. Although most chemical screens described to date are using embryos less than 72 hpf, there are examples of chemical screens performed in older embryos up to 20 dpf with soaking technique.[34] Thousands of wild-type or genetically modified embryos can be obtained from a large mating, ideal for chemical screens, both genetic and phenotypic.[35] In a given genetic or disease model, a chemical suppressor screen can be performed in an attempt to reverse the phenotype or the genetic lesion, identifying new pathways of regulation in the pathophysiology of disease.

The zebrafish has been an ideal platform for *in vivo* drug discovery with new small-molecule libraries, testing FDA-approved drug libraries for new indications or biologically active compounds with unknown effects.[36] *In vivo* drug screening has the advantage of avoiding drug toxicities and off-target effects that cannot be assessed in *in vitro* or cell culture model systems.[37] One disadvantage of such screens has been a whole-embryo effect of the drug, making it difficult at times to distinguish the exact target of the drug in a given context. Thus, validating the positive hits from such screens in other model systems has been essential.

One of the most successful compounds that has so far been identified from a chemical screen in a zebrafish is prostaglandin E2 (PGE2), which has made its way to a human clinical trial of umbilical cord transplantation. PGE2 was identified in one of the initial chemical screens performed in the hematopoietic system to identify compounds that affected *runx1*$^+$*cmyb*$^+$ HSPCs in zebrafish.[38] Three compound libraries were used: NINDS Custom Collection of 1040 compounds, SpecPlus Collection of 960 compounds, and BIOMOL ICCB Known Bioactives of 480 compounds. About 5% of these compounds were toxic to the zebrafish embryos, causing death or severe morphologic abnormalities. The screen identified two compounds, linoleic acid and celecoxib, that had opposite effects on the HSPC population—increasing or decreasing their number in the CHT, respectively. Both of these compounds acted on prostanoids and their effector PGE2. PGE2 by itself was able to increase the number of *runx1*$^+$*cmyb*$^+$ HSPCs in both embryos and adult fish in a transplantation setting. Furthermore, exposure to PGE2 for only 2 hr enhanced the long-term reconstitution potential of murine HSPCs. Most importantly, 16,16-dimethyl prostaglandin E2 (dmPGE2), the metabolically active derivative of PGE2, is currently being tested in a human trial to enhance umbilical cord transplantation. The number of HSCs with reconstitutive ability in a single umbilical cord unit has limited its use in adult recipients of stem cell transplantation who, because of their size, require double-umbilical cord units to ensure engraftment. More than 85% of the time in double-umbilical cord transplants, the chimerism quickly is skewed toward one of the two units.[39] An early report of the human trial showed that dmPGE2-treated umbilical cord units had preferential long-term engraftment in 10 of 12 treated participants.[40] Currently, studies are underway to understand the mechanism by which PGE2 affects the HSPCs.

Zebrafish was used to perform the first chemical screen in transplantation biology, using ICCB Known bioactive library from BIOMOL of 480 compounds. This screen was done using isolated whole kidney marrow cells, and the readout was relative HSPC activity in competitive transplantations in a transparent zebrafish line called *casper*.[41] GFP and DsRed2 transgenic fish lines were used as donors, and the ratio of GFP/DsRed2 intensity was measure both by direct imaging and by flow cytometric analysis of the recipients' kidney marrow after conditioning with irradiation. Two compounds, not previously implicated in HSPC biology, were identified: 11,12-epoxyeicosatrienoic acid (11,12-EET) and 14,15-EET, which are eicosanoids synthesized through the cytochrome P450 epoxygenase pathway. One such enzyme, Cyp2j6, has been reported to be enriched in murine long-term HSCs.[42] The mechanism of action of EETs or the role of epoxygenase pathway has been largely unknown in HSPCs. The zebrafish studies with EET showed that treatment with 11,12-EET at the time of definitive hematopoietic initiation increased *runx1*$^+$ HSPCs in the AGM, suggesting that it acts at the level of hemogenic endothelium promoting the hematopoietic program in these cells. Molecular dissection of the mechanism by which this is orchestrated showed that multiple activator protein 1 (AP-1) family transcription factors, like *fosl2, junb,* and *junbl*, orthologs of human JUNB, were upregulated upon 11,12-EET treatment. AP-1 program activated *runx1* expression, sealing the signature of HSC fate in the hemogenic endothelium. AP-1 has also been shown to be important in cell–cell signaling critical for cell migration, thus possibly explaining its role and the positive effect of 11,12-EET on HSPC migration and homing.[41] It is interesting that PGE2 and EET, two small inflammatory lipids, have potent effects on stem cell migration and engraftment. Enhancing engraftment of HSCs after transplantation is of utmost clinical importance to shorten the period of time of neutropenia and transfusion dependence, in an effort to reduce stem cell transplantation-associated morbidity and mortality. Thus, there is great interest in bringing the EET family of compounds to clinical trials.

LIVE IMAGING OF HEMATOPOIETIC STEM AND PROGENITOR CELLS

Studying biology over time and in motion in its native environment is a treasured goal in life sciences. Despite the elaborate and detailed definition of murine HSPCs *via* various combinations of surface marker expression, imaging of these cells in their native niche, the bone marrow, has been

challenging, and mostly depended on static sections.[43,44] The transparency of the zebrafish embryos combined with the advances of live cell imaging has made it possible to analyze the hematopoietic development, HSPC migration, and interactions with their environment at the cellular level. The limitation in the field of zebrafish hematopoiesis is the relatively broad definition of HSPCs compared with murine or human HSPCs, which are characterized with more specific surface immunophenotype.

The approaches in live animal imaging of zebrafish embryos have used various fluorescent transgenic lines. These lines express fluorescent proteins under HSPC-specific promoters such as *c-myb, cd41,* or *runx1.*[45–47] Live imaging of these transgenic lines has provided detailed description of the birth of the HSPC from the aortic floor and the AGM. By labeling the HSPCs by *c-myb:eGFP* or *cd41:GFP* and the endothelial cells with *flk1:memcherry, flk1:GFP,* or *lmo2:dsred*, it was shown that the HSPC directly emerged from the aortic endothelial cell. The hemogenic endothelial cell expresses the HSPC marker cmyb, and then it undergoes a special movement of bending toward the subaortic space and eventually is released into the circulation.[45] More recently, HSPC behavior and migration was documented using another transgenic line, *runx1:GFP* or *runx1:mcherry*, to visualize migration of HSPCs through the CHT, the caudal hematopoietic tissue, which is a transient residence of these cells between their birth in the AGM and their final destination in the kidney marrow of the fish. Runx1:EGFP^{+} HSPCs were imaged using spinning disk confocal microscopy for up to 16 hr, and were observed to exit the circulation from the dorsal aorta or the intersegmental vessels and lodge just outside of the endothelial cells. Within minutes of this exit, the HSPC would be surrounded by a group of endothelial cells, actively changing to form a space or a pocket for the HSPC, a process termed "endothelial cuddling."[48] Using this model, it was also shown that stromal cells, expressing DsRed2 under the *cxcl12a* promoter, also interacted with the extravasated HSPC, and helped orient the cell during cellular divisions. The HSPC in close contact with the stromal cells would either not divide, or divide with both cells interacting with the stromal cells, possibly resulting in a self-renewing cell division. Lastly, the HSPC division at an asymmetric orientation with the stromal cell would result in one daughter cell remaining in close proximity with the stromal cell, while the other daughter cell detaching from the stromal cells, and going back into the circulation. The effects of the niche cells on HSC functions are of great interest in the field of hematopoiesis, and live cell imaging has facilitated those studies greatly. This system has also been valuable in identifying biologically active compounds *via* chemical screens that would enhance the interaction of HSPC to the endothelial cells facilitating engraftment of HSPCs in its niche.

An innovative model of elucidating HSPC and niche interaction has been created by combining transgenic fish lines and live imaging in parabiotic zebrafish, where two fish are allowed to grow after an early fusion step during blastula stage, and share circulating cells.[49] This system provides a powerful method to study the effects of genetic lesions on the niche, to isolate the cell-autonomous and non-cell-autonomous variables in HSPC biology. Combining a wild-type and mutant zebrafish embryos and growing them into early larval stages, one could study the effect of wild-type environment on mutated HSPCs or wild-type HSPCs in the mutated niche. As the parabiotic fish share a common blood stream and circulating soluble chemicals are also shared,[49] this model is also useful in studying the effect of overexpressed cytokines or other circulating signals on hematopoiesis.

Other live imaging techniques have utilized the *casper* zebrafish with transparent adult animals that could be easily imaged. Capability of imaging *casper* fish, however, is limited by the semiquantitative nature of the signal and the requirement of the signal to be close to the body surface.[50] A recently described method utilized wild-type fish and provided a method of more accurately quantifying *in vivo* luminescence while minimizing signal-to-noise ratio. The zebrafish named "zebraflash" is a luciferase-based transgenic line that can provide deep tissue bioluminescence information in a freely swimming organism.[51] This model has been used to track HSPC transplantations in zebrafish and can be utilized to track the migration and the behavior of both the stem cell and its progeny over time.

REVERSE GENETIC MODELS TO STUDY HEMATOPOIETIC DISORDERS

The zebrafish genome, first published in 2002, and later modified and expanded in 2013,[52] showed that 70% of human genes have a zebrafish ortholog. This has been a critical finding for utilizing the zebrafish for reverse genetic studies in hematopoietic disorders. The role of a gene in a particular phenotype can be easily assessed by genetic manipulation of zebrafish embryos with microinjections of mRNA for overexpression of the

gene, morpholino antisense oligomers, or guide RNAs for Cas9-mediated site-specific genomic targeting.[12,53] With tissue-specific Cas9 expression, it is now possible to perform gene editing on any gene of interest even more precisely in a defined hematopoietic lineage rather than the whole embryo.[33]

For example, a zebrafish model of Diamond–Blackfan anemia (DBA) has helped to elucidate a mechanism by which the underlying ribosomal protein mutations lead to the disease in humans. Two reports found that knockdown of ribosomal protein 19 (rps19) leads to a significant loss of erythrocytes in zebrafish, in agreement with the human phenotype of pure red cell aplasia in DBA patients.[54,55] In zebrafish, it was found that this mutation results in a differentiation block in the erythroid lineage and subsequent apoptosis of the red cells mediated by p53. Blocking the p53 pathway partially rescues the phenotype seen in rps19 knockdown fish. This finding opened novel avenues of research to further clarify the mechanism of anemia in DBA, and it also provided potential therapeutic targets in the p53 pathway.

In the field of hematopoietic malignancies, various oncogenes have been utilized to model T-cell acute lymphoblastic leukemia (T-ALL) and T-cell lymphoblastic lymphoma (T-LBL). T-ALL was the first hematologic malignancy modeled in zebrafish by overexpressing the mouse *Myc* gene under the control of *rag2* promoter.[56] The disease recapitulated the human condition, and in addition provided a way of tracking and visualizing the leukemia cells as the *Myc* gene was tagged with enhanced GFP as well. The T-LBL model was based on a heat-inducible *Myc* expression, and over time progressed to T-ALL.[57] This transformation is known to occur in humans as well, but the mechanism of a localized lymphoma in the thymus spreading to the blood in a form of leukemia has been unknown. The fish model using the heat-inducible oncogene showed that part of the regulatory mechanism of the cancerous cells within a lymphoma is mediated by apoptosis regulator bcl2, sphingosine 1-phosphate receptor 1 (s1p1), and intracellular adhesion molecule 1 (icam1), which together inhibit apoptosis and prevent cell–cell interactions required for vascular invasion and spread. Upon Akt activation, however, this block is overcome and the cells are able to seed the circulation, transforming into T-ALL.

Models of acute myeloid leukemia (AML) and myeloid disorders have been successful in zebrafish as well. Overexpression of a common oncogenic protein AML1-ETO, resulting from a translocation found in AML, had limited utility because of early lethality in fish.[58] The heat-shock-inducible protein somewhat resembled but did not quite phenocopy the AML in adult fish. However, it provided important mechanistic information about how this protein may act. It was found that AML1-ETO induction leads to cellular fate change in erythroid cells to more myeloid lineage and that the transcription factor *scl* was required for this switch. Recently, the first zebrafish models of myelodysplastic syndromes (MDS) and myeloproliferative neoplasms (MPN) were reported.[59,60] Gjini and colleagues studied zebrafish with homozygously targeted *tet2* mutation often found in MDS. The authors used zinc finger nuclease technology to establish a stable zebrafish line with loss-of-function mutations in the *tet2* gene and characterized a phenocopy of the human MDS condition in these fish after 24 months, with cytopenias and dysplastic cell populations in the kidney marrow.[59] *Tet2* mutations alone in human patients often result in clinically benign clonal hematopoietic proliferation, and require secondary and sometimes tertiary mutations for progressing into clinical apparent MDS/AML. Given the length of time the *tet2*-mutated state required to have apparent manifestation, it is possible that secondary mutations could have been acquired, similar to the pathophysiology of MDS in humans. The MPN-like model in zebrafish was identified as a result of a mutagenesis screen using ENU, which identified a zebrafish line that had increased HSPCs and differentiation into myeloid lineages. The *c-cbl* gene, an ortholog of c-CBL implicated in human MPN, was found underlying this mutant line.[60] The *c-cbl*-mutated zebrafish line depended on flt3 signaling for its phenotype, validating the similarity with MPN in human patients.

CONCLUSIONS

Zebrafish has become an invaluable vertebrate model for studies in hematopoiesis over the past four decades. From developmental biology to modeling hematopoietic disorders, various aspects of hematopoiesis have been studied in zebrafish to date, and the limits of what is possible are expanding constantly. New innovative technologies, such as state-of-the-art microscopes, novel gene targeting or gene knock-in with the CRISPR-Cas9 system, and improved transplantation assays, join the well-established and unique approaches of forward genetic and chemical screens, making the zebrafish an even more attractive platform to study human diseases.

There are some technological limitations, however, that, if overcome, would further improve zebrafish as a model system. In contrast to the mammalian system, there are very few antibodies available for immunophenotypical identification of HSCs or other hematopoietic cells. Recently, promising surface marker cd41 was identified to define a population of cells that contains the stem cell activity in the kidney marrow in adult zebrafish.[61] Additionally, studies describing MHC class I loci have been instrumental in solving the issue of multiple polymorphisms in wild-type zebrafish lines. Characterization of zebrafish MHC loci will provide immunologically compatible donor and recipient organisms to enhance transplantation assays, and will be critical for more rigorous experimental designs in matched genomic backgrounds.[62,63] Another limitation in the field of zebrafish hematopoiesis studies has been the lack of *in vitro* culture system for growing and differentiating HSCs. Two successful stromal lines have been described that support HSPCs: zebrafish kidney stromal cells (ZKS)[64] and zebrafish embryonic stromal trunk (ZEST) cells.[65] However, currently no cell-free model system exists for culturing HSPCs with defined growth factors.

The steadily growing zebrafish research community continues to harvest the potential of zebrafish to expand our understanding of hematopoiesis and its disorders, and to discover new drugs to treat these diseases.

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