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USING THE IN SITU HYBRIDIZATION TECHNIQUE TO EXEGETICS THE ZEBRAFISH HEMATOPOIESIS

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ABSTRACT

The zebrafish (Danio rerio) has developed as an idyllic creature for the analysis of hematopoiesis, the procedure by which every cellular elements of the blood are generated. These major essentials including erythrocytes, granulocytes, monocytes, lymphocytes and thrombocytes are typically generated by complex genetic signaling paths that are extremely preserved during phylogeny. Three dissimilar *mon* mutant alleles each encode premature stop codons, and enforced expression of wild-type *tif1γ* mRNA rescues embryonic hematopoiesis in homozygous *mon* mutants. Amazingly, higher level of zygotic *tif1γ* mRNA expression explains ventral mesoderm during hematopoietic stem cell and progenitor creation preceding to *gata1* expression. Transplantation readings clearly reveal that *tif1γ* functions in a cell-autonomous manner throughout the differentiation of erythroid precursors. Research works in murine erythroid cell lines validate that Tif1γ protein is localized within original nuclear foci, and the expression typically decreases during erythroid cell maturation. The results or this research work establish a key role for this transcriptional intermediary factor in the differentiation of hematopoietic cells in case of vertebrates. In this paper, using the In Situ hybridization technique to exegetics the Zebrafish hematopoiesis has been clearly analyzed and elucidated.

Keywords: Zebrafish, In situ hybridization, hematopoiesis, transcription factors, Micro RNA and mesoderm.

INTRODUCTION

The tropical fish pet, the zebrafish (*Danio rerio*) has now converted into a dominant vertebrate exemplary for the analysis of hematopoiesis and several other aspects of embryogenesis and organogenesis. Contrasting with mammals, zebrafish eggs are fertilized outwardly and are voluntarily obtainable for observation or handling even from the single-cell embryo stage. The embryos are optically visible, so the complete organism can be effortlessly assessed beneath a dissecting microscope, including direct visualization of the heart beat and circulating blood cells in the vasculature. These minor animals attain sexual maturity merely in three to four months, and the adult females are viable in producing hundred to two hundred eggs for every week. Numerous thousands of animals can be reserved in a fish facility requiring abundant lesser space than the mice or other supporting mammals, and therefore the zebrafish is a profitable experimental vertebrate model for extensive genetic screening. Though invertebrate models have been priceless towards the study of embryogenesis, these organisms are not beneficial for the analysis of hematopoiesis or the function of mature blood cells. Using the zebrafish, numerous significant forward genetic screens in the past few decades have produced thousands of mutants, several with hematopoietic defects that have boosted the understanding of all necessary features of hematopoiesis. Every vertebrate, including bony fish (teleosts), possess two waves of hematopoiesis. The former of these is referred as the primitive or embryonic hematopoietic wave, and principally generates erythrocytes, as well as some embryonic macrophages. In mammals and birds, this first hematopoietic wave is observed in the extraembryonic yolk sac where early erythrocytes are generated (Al Adhami *et al.* 1997, Amatruda *et al.* 2002).

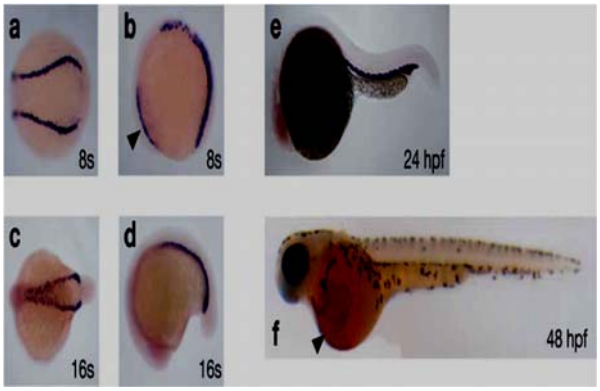

Figure-1. Creation of early blood precursors in zebrafish embryo. (a) and (b) Whole-mount in situ hybridization with scl at the 8 somite stage, marking bilateral stripes of lateral plate mesoderm which would ultimately migrate medially and fuse to form ICM. Anterior hematopoietic precursors are also obvious in the RBI (arrow). (c) and (d) Whole-mount in situ hybridization with gata-1 at 16 somite stage. The anterior section of the lateral plate mesoderm is commencing to fuse, displaying the creation of ICM. (e) Whole-mount in situ hybridization with gata-1 at 24 hpf, marking erythroid precursors in ICM just prior to the onset of circulation. (f) o-dianisidine staining of hemoglobin at 48 hpf in circulating the erythrocytes, observed conspicuously in the ducts of Cuvier over the yolk sac (arrow). (b), (d), (e), and (f) display lateral interpretations of the embryos, with the anterior to the left. (*a*) and (*c*) display dorsal views of the embryos (Hisa *et al.* 2004).

In zebrafish, the primitive hematopoiesis happens in two intraembryonic sites: the intermediate cell mass (ICM) which is situated in the trunk ventral to the

notochord, and the rostral blood island (RBI) ascending from the cephalic mesoderm. Inside the posterior mesoderm, cells lateral to the developing somites define both vascular and blood markers and migrate medially around 18 h post fertilization (hpf) to fuse at midline forming the ICM as depicted in figure 1. Cells inside the ICM, corresponding to the mammalian yolk sac blood island, discriminate into the endothelial cells of the trunk vasculature and proerythroblasts, which start to arrive the circulation around 24 hpf. Simultaneous with the primitive erythropoietic wave in the posterior ICM, the cells in the anterior mesoderm of the zebrafish embryo create a second anatomical site for hematopoiesis, typically referred as the RBI, which mainly generates macrophages as discussed in the subsequent sections.

ZEBRFISH (DANIO RERIO) AS A MODEL ANIMAL

Zebrafish is an overwhelming model creature to study both developmental and physiological processes, because of the well-known series of characteristic features, encompassing its sequenced genome (Barbazuk et al. 2000). There are supplementary benefits in using zebrafish as a comprehensive model animal. Zebrafish classically became one of the most rampant and deep-rooted models in genetics and developmental biology owing to its smaller life cycle, minor size and ease in case of laboratory maintenance. These features highlighted zebrafish as a convenient model in cancer research including the chemical carcinogenesis (Bartel, 2009). Zebrafish has also developed the importance of a principal research exertion into permissive to the molecular and cellular events which generally command the development of vertebrate embryos. Equally, the zebrafish has revealed good-looking in studies scrutinizing the issues which distraught the formation of transgenic fish and the expression of transgenes. The developments which have been made in these sections have unwaveringly established this trivial aquarium fish as a foremost model system in biological and biotechnological research activities (Beckwith *et al*. 2000, Bennett *et al*. 2001, Kanki et al.2001, Best *et al*. 2002, Brownlie *et al*. 1998, Cammas *et al*. 2000, Cammas *et al*. 2002, Chen *et al*. 2009).

MATERIALS AND METHODS

Assortment of targets and read-outs

The most imperative choices in planning a zebrafish chemical screen is to pick the suitable targets and read-outs. The nominated target and read-out should precisely replicate the desired procedure, and should also be vigorous, reliable and sensitive. ISH can be employed to visualize the general expression of any gene product. This analyze necessitates preceding idea about the biologic process and depends on the nominated molecular target, which must be precarious for the developmental process. This assay also needs the development of gene specific antisense probe and optimization of ISH circumstance. The sustenance procedure in this paper describes the design and combination of an antisense probe. If a suppressor screen is done, predominantly for suppressors of a homozygous recessive mutation, greater penetrance of ISH staining phenotype in the mutant embryos is required, which might require the assessment of numerous dissimilar molecular targets. Apart from ISH for molecular targets, some other assays have been employed effectively in the previous screens. For instance, a visible morphological trait links with the developmental procedure like the D-V axis of the developing embryos has been used a read-out. This particular type of assay does not necessitate much preceding information about the procedure, but the phenotypes may not be precisely linked to the biology, and several structures like internal organs are not effortlessly assayable. A tissue or pathway-specific reporter transgenic zebrafish are suitable for fluorescent imaging-based high-throughput screen, but they frequently require the probing for a suitable promoter and generation of stable transgenic lines.

Duration of chemical treatment

The suitable duration for chemical action is influenced by the targeted gene or biological procedure. Commonly, chemical actions should commence prior and should continue until the expression of targeted gene or the existence of interested process. For instance, to assess the HSC development and requirement in zebrafish, the embryos are generally treated from 3 somite stage for about 36 hours post fertilization (hpf).

In another screen searching for signals variable HSC migration and mobilization to the caudal hematopoietic tissue (corresponding to mammalian fetal liver), zebrafish embryos are preserved from about 48 hpf to 72 hpf.

The Zebrafish Information Network (ZFIN) is a respected online resource that delivers a complete gathering of gene expression information for multiple developmental time points. Additional factor to keep in mind is the toxicity; younger embryos are more susceptible to the harmful things of some chemicals. In our knowledge, treatment before gastrulation stage (10 hpf) and predominantly before 50% epiboly stage (6 hpf), suggestively rises the death or the developmental delays of embryos as associated with the treatment after gastrulation finishes. The initial treatment can also inhibit with the developmental planning during embryogenesis that will probably affect the gene expression, and consequently complexes the interpretation of results.

Protracted disclosure to the chemicals (more than 24 hours) is also poisonous to the embryos (Detrich *et al.* 1995, Friedman *et al*. 1996, Fujiwara *et al*. 1996). If the assay is read out at the late developmental phases, a wash-out step may be added to eliminate the chemicals after the preliminary treatment. The effects from the early chemical treatment should be checked during the succeeding development phases.

Selecting a chemical library

Many chemical libraries are existing from minor collections of characterized compounds with recognized molecular targets to superior libraries comprising of tens of thousands of compounds of unidentified function. A cautious overview and comparison of dissimilar libraries have been deliberated elsewhere. One should pick the library based on the intended pathways, the throughput of the assay and cost of the library. Chemical libraries with branded compounds are normally used in zebrafish small molecule screens. Once a list of authenticated hits is created, the continuation and mechanistic studies can be speedily performed. Supplementary single commercial chemicals can be bought to further learning pathways that seem noteworthy during the screen. If a chemical library with a great quantity of chemicals is designated, pooling of chemicals can be used to enable the haste of the screen, which has been effectively used in the minor molecule suppressor screen of zebrafish bmyb mutants. But a chemical pool may consequence in unanticipated interactions amongst pooled chemicals and also augmented toxicity to the embryos. This is predominantly factual for libraries with bioactive compounds. Additionally, one should prudently choose the pooling approach. For instance, as in the aforementioned screen, every chemical is screened twofold in two dissimilar chemical pools.

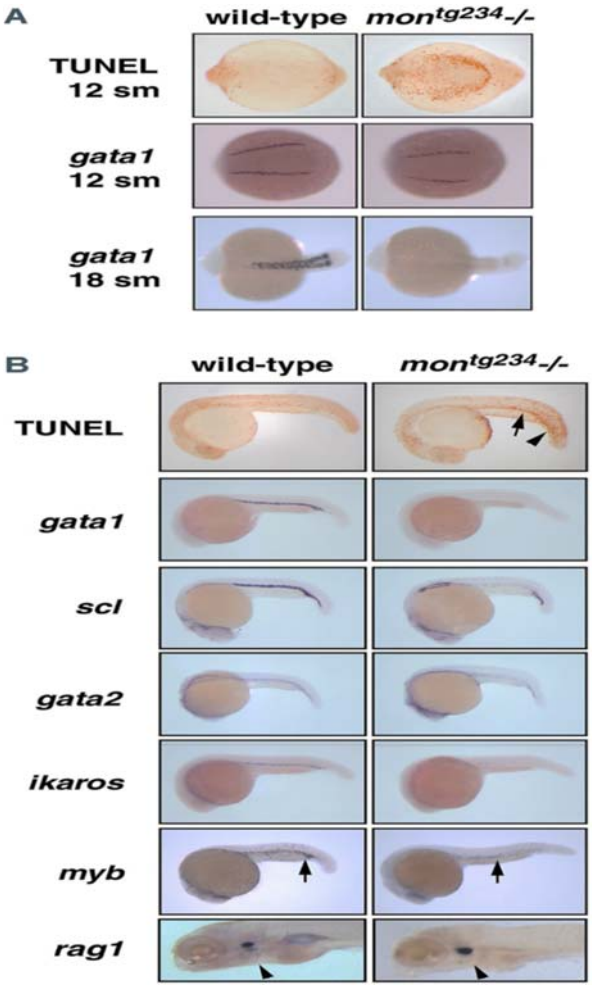

Figure-2. Zebrafish *mon* Mutants have severe imperfections in Primitive Hematopoiesis. (A) Whole-mount TUNEL assays expose that ventral-posterior mesodermal cells suffer apoptosis in homozygous *mon*^{tg234} mutant embryos. Whole-mount in situ hybridization of *gata1* perceived at the 12- and 18-somite phase in genotyped embryos. Posterior sights, anterior to the left. (B) Widespread apoptosis is noticeable in the trunk and tail (arrowhead) and also in hematopoietic cells of the embryonic blood island typically at 22 hours of development (arrow). Whole-mount in situ hybridization at 22 hpf including *scl, gata2, gata1, ikaros,* and *myb* in *mon*^{tg234} mutants. Expression of *myb* is significantly abridged in the blood islands because of a loss of erythroid cells, but embryonic macrophages are still existing (arrows). The expression of *rag1* in thymic T-cells seems usual in *mon* mutants at 5 d post fertilization (arrow heads).

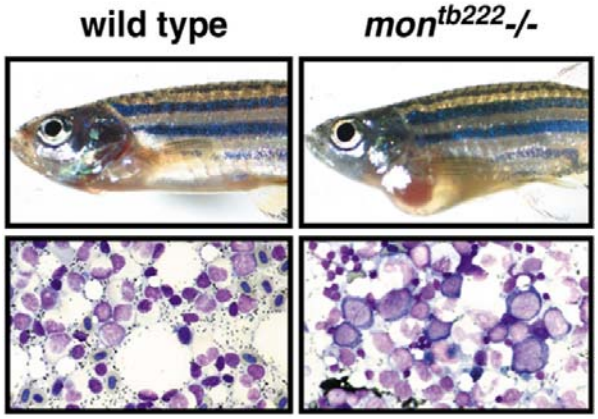

Figure-3. Zebrafish *mon* mutants also have severe imperfections in Definitive Hematopoiesis: Adult phenotype of wild-type and *mon* mutants. A sporadic surviving mon^{tb222} homozygous adult displays noteworthy cardiomegaly in assessment to a wild-type age-matched control. Wright–Giemsa stained marrow of wild-type adult in assessment to a homozygous mutant. Note the dramatic lessening of terminally differentiated erythroid cells and the occurrence of abnormally huge megaloblastic proerythroblasts in the mon^{tb222} mutant marrow.

In order to conclude when the *mon* gene is mandatory in development, we first examined hematopoietic gene expression and apoptosis in the zebrafish homozygous *mon* mutant embryos. Throughout embryogenesis, homozygous zebrafish *mon* mutants have no red blood cells (RBCs) noticeable in circulation. The *mon* mutants initiate expression of *gata1* in the hematopoietic cells round the five-somite stage, comparable to wild-type embryos; nevertheless, based on TUNEL staining, the distinguishing erythroid cells experience programmed cell death from the 12-somite stage to 22 h postfertilization (hpf) (2A and 2B, arrows). At 12 somites, *gata1* expression is only marginally reduced. By 18-22 hpf, hematopoietic-specific markers such as *gata1, scl, gata2,* and *ikaros* are not noticed in the embryonic blood island (2A and 2B). The hematopoietic cells are thus appropriately quantified early during the development of *mon* mutant embryos, but these precursors experience cell death. Based on expression of c-*myb* and *rag1* (2B, arrows), *mon* mutants have usual myeloid and lymphoid development, correspondingly. In addition to the shortage of RBCs in *mon* mutants, there is a protruding loss of fin-fold and tail mesenchyme. TUNEL staining of *mon* mutants reveals extensive apoptosis of mesenchymal cells in the trunk and tail bud areas (2A and 2B, arrows). The *mon* gene is thus essential for normal development and existence of both committed erythroid progenitor cells and the posterior mesenchymal cells.

Also we have inspected definitive hematopoiesis in rare surviving homozygous adult zebrafish *mon* mutants. Mutations in *mon* are commonly lethal by 10 to 14 d of development, though rare *mon* homozygous mutants (approximately 1 in 500 bloodless embryos) of all tested alleles live to adulthood. Adult *mon* mutants display cardiac hypertrophy, apparently due to the unadorned anemia leading to a great output state (Figure-3). In wild-type zebrafish, the adult site of hematopoiesis is the kidney, which encompasses erythroid, lymphoid, and myeloid populations at several phases of differentiation. In *mon* homozygous mutants, there is an unadorned block in maturation at the proerythroblast stage (Figure-3), however the differentiation of myeloid cells is usual. This validates that the *mon* gene product acts during both the primitive and definitive erythropoiesis.

RESULTS

Positional Cloning mechanism recognizes the *mon* as the Zebrafish Ortholog of Mammalian TIF1γ

To agree whether *tif1γ* is conveyed in hematopoietic mesoderm, we subsequently inspected zebrafish embryos by whole-mount in situ hybridization (Figure-4A). *tif1γ* mRNA is articulated maternally and is found throughout the embryo during blastula stages. Throughout gastrulation and epiboly stages, zygotic expression of *mon* is maximum in the mesendoderm of the germ ring. At tail bud and initial somite stages a great level of *tif1γ* expression describes a horseshoe-shaped population of ventral/lateral mesoderm that will yield to blood and also expresses *stem cell leukemia*hematopoietic transcription factor *(scl)*.

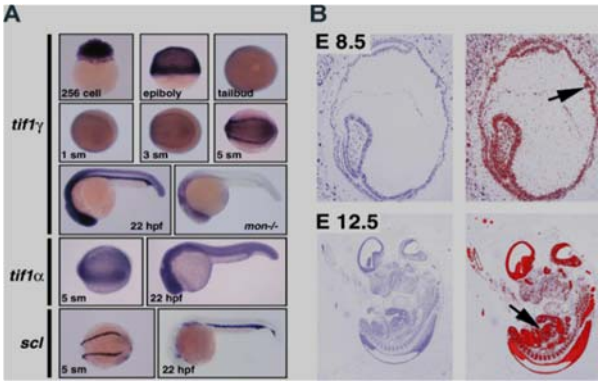

Figure-4. The *mon/tif1γ* Gene is highly expressed in Hematopoietic Mesoderm: (A) In situ hybridization of zebrafish embryos signifying the embryonic expression of *tif1γ. tif1γ* is originally expressed as a maternal mRNA. Enlarged expression of *tif1γ* in ventral-lateral mesoderm commences amongst the one- to three-somite stages and rises through early development. By five somites, *tif1γ* is intensely expressed in lateral stripes of mesoderm that also express *scl.* At 22 hpf *tif1γ* is expressed largely in the brain, spinal cord, trunk and tail mesenchyme, but is at much greater levels in hematopoietic cells of the blood island. Zebrafish *tif1α* is also largely expressed but moderately more uniform in most tissues, in contrast with *tif1γ. Tif1α* is feebly expressed at early somite stages in hematopoietic mesoderm and consistently expressed at 22 hpf, including expression in the blood islands. Expression of *scl* at five somites and 22 hpf specifies the

embryonic blood island in comparison to *tif1γ* expression. (B) In situ hybridization of mouse embryos notices broad expression of *Tif1γ* at embryonic day 8.5 comprising of the yolk sac blood islands (arrow). AT embryonic day 12.5, there is great level expression in the fetal liver (arrow) and wide expression in the embryonic brain, spinal chord, gut and muscle.

This group of cells endures to express *tif1γ* and *scl* while it converges and forms the embryonic blood island. The *tif1γ* gene is also extremely expressed in the central nervous system as well as the mesenchyme of trunk and tail. Homozygous *mon*^{tg234}mutants have a significantly concentrated amount of *tif1γ* mRNA in all tissues consistent with nonsense-mediated message decay. Consequently, zebrafish *tif1γ* is explicitly expressed in ventral mesoderm and putative hemangioblasts prior to and throughout the embryonic stages when hematopoietic progenitors are experiencing apoptosis in *mon* mutants. We also associated the expression of zebrafish *mon* to mouse *Tif1γ* (Figure-4A and4B). Mouse *Tif1*γ is exceedingly expressed in erythroid blood islands of the yolk sac, and it is successively expressed in the fetal liver at a great level, and in other tissues, comprising of the central nervous system. Taken together, these results intensely suggest that zebrafish *mon* and mouse *Tif1γ* are orthologs that function during the hematopoiesis.

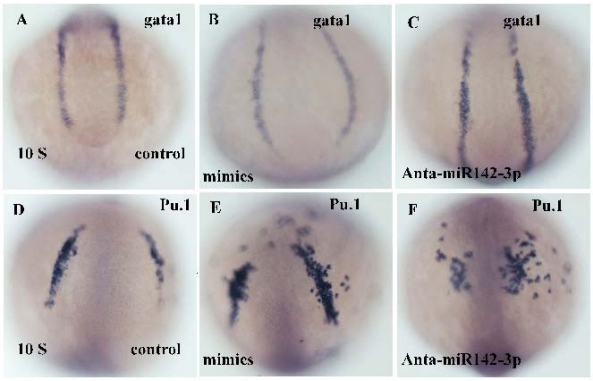

Figure-5. Gata1 and pu.1 in situ Hybridization results at 10 somite stage. (A) control, (B) gata1 marker expression at 10 somite stage displayed that miR142-3p (mimic) over expression caused lessening in erythroid cells progenitors formation, while (C) antagomir caused knock down this reduction to show increase in erythroid progenitors formation associated with (A). At the same stage, (D) control, (E) pu.1 marker expression displayed that miR142-3p (mimic) over expression caused rise in myeloid progenitors cells formation, by dissimilarity (F) antagomir142-3p cut myeloid progenitors cells and both of them miR142-3p (mimic) and antagomir produced migration disorder for myeloid progenitor cells when associated with (D) (Kim et al. 1996).

Also Whole-mount In Situ Hybridization (WISH) examination for 24 hpf injected embryos exhibited with gata1 marker expression that miR142-3p suppress expressively erythroid progenitors formation and Antagomir142-3p knockdown and this repression as exhibited in figure (5-a, b, c), and myeloid pu1 marker is mandatory for zebrafish myelopoiesis thereby it exhibited increase in myelopoiesis and antagomir knockdown, this rise to show lessening with myelopoiesis as displayed in Figure (5-d, e, f).

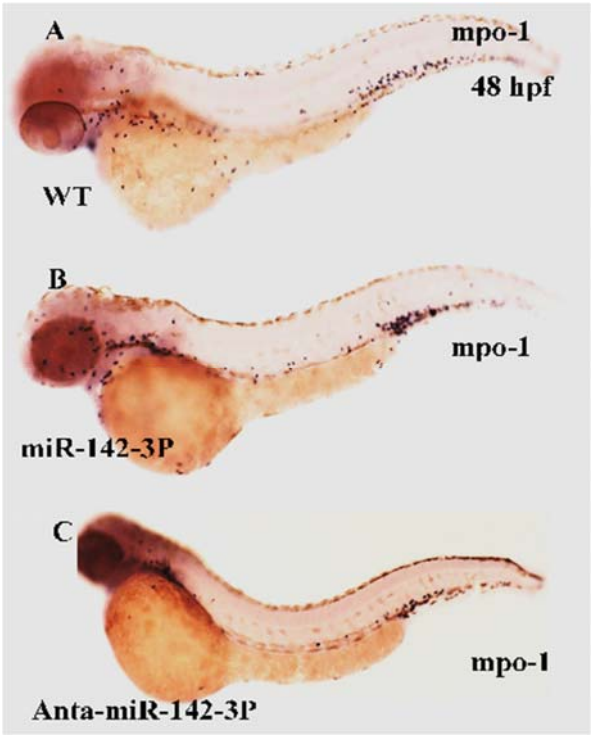

Figure-6. Mpo-1 marker in situ Hybridization results at 48 hpf.(A) control, (B) agomir142-3p over expression caused significant increase in granulocyte formation, and (C) antagomir142-3p showed decrease this granulocyte formation, compared with (A).

The transcription factor c-myb has developed as one of the significant regulators of vertebrate hematopoiesis. In mice, it is dispensable for primitive stages of blood cell development but fundamentally mandatory for definitive hematopoiesis, so here also we have exasperated to know whether miR142-3p is obligatory for definitive hematopoiesis, we employed runx-1 and cmyb markers and the results revealed that miR142-3p is not essential for definitive hematopoiesis as exhibited in Figure-6.

Forced expression of *tif1γ* releases the Hematopoiesisin *mon* Mutants

To further approve that a mutation in the zebrafish *tif1γ* gene is accountable for the *mon* mutant phenotype we accomplished embryo rescue experiments (7A). Microinjection of synthetic wild-type *mon* mRNA at the one-cell stage releases the creation of embryonic erythrocytes in genotyped mutant embryos without

producing apparent imperfections in embryonic patterning or organogenesis. At 4 d of the development, 70% (n = 10) of *mon*^{tg234} mutants display noteworthy (greater than 200 cells in contrast to a wild-type estimation of 3,000 cells) rescue of circulating hemoglobinized RBCs in assessment to control sibling mutants (n = 75). Based on the correction of the jagged fin-fold phenotype, the mesenchymal cells are rescued to a comparable extent as the anemia. Overexpression of *mon* did not effect in expanded blood cell quantities in wild-type embryos and was not toxic at doses that rescue the phenotype of *mon* mutants. Meanwhile, there were no expanded or ectopic blood populations in the embryos, these rescue experimentations propose that *mon* functions as a permissive factor essential for hematopoiesis (Rodriguez *et al*. 2004).

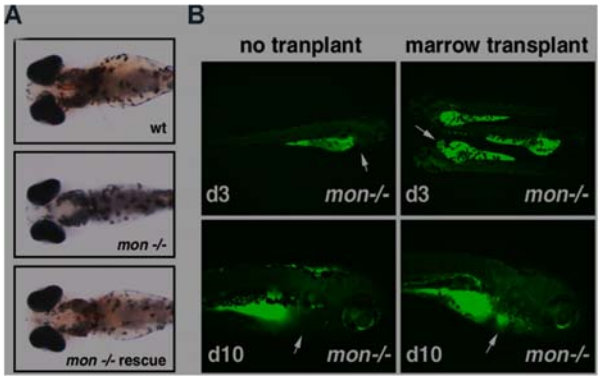

Figure-7. Over expression of Wild-Type *tif1γ* mRNA or Marrow Transplantation releases Embryonic Hematopoiesis in *mon* Mutants. (A) *mon*^{tg234} mutants are released by injection of mRNA-encoding wild-type Tif1γ protein. At 4 d of development, great quantities of RBCs are noticeable in the circulation of wild-type zebrafish, displayed here by o-dianisidine staining of hemoglobin. Uninjected *mon*^{ttg234} homozygous mutants are entirely bloodless. Injection of 100 pg of wild-type *tif1γ* mRNA releases erythropoiesis in mutant embryos. o-dianisidine-stained larvae are displayed in ventral views to highlight blood in vessels. (B) Transplantation of wild-type zebrafish marrow cells that are typically carrying a *gata1: GFP* transgene into the 2-d-old embryos reconstructs erythropoiesis, but not sustainability, in *mon*^{tg234} homozygous mutants. Still frames from movies of live embryos at day 3 post-transplant highlight fewer than 100 GFP$^+$ RBCs in circulation (top). Transplanted cells were perceived to proliferate resulting in thousands of donor-derived erythrocytes 7 d later (bottom). Arrows specify the hearts of control and transplanted zebrafish.

DISCUSSIONS

As part of a large-scale forward genetic screen, we formerly recognized a complementation group of self-regulating mutant alleles in the zebrafish gene that we termed as *moonshine*. Positional cloning was used to recognize the *mon* gene, forming a serious role for a transcriptional intermediary factor, Tif1γ, throughout hematopoietic development.

The *mon* gene encodes the Zebrafish Ortholog of Mammalian TIF1γ

Our results intensely provision the supposition that we have positionally cloned the zebrafish *mon* gene appropriately, and it is the ortholog of mammalian Tif1γ. Tif1γ is existent in the critical genetic interval encircling a single approximately 50-kb PAC clone well-defined by linkage examination. Sequence analysis specifies that zebrafish *tif1γ* is most analogous in predicted amino acid sequence and intron/exon construction compared to the predicted orthologous human and mouse genes. Zebrafish *tif1γ* is typically positioned in general region of zebrafish Chromosome 8 syntenic to the region of human Chromosome 1 comprising *TIF1γ*. We recognized point mutations in *tif1γ* from three dissimilar alleles of *mon* that each outcome in premature stop codons and mRNA decay. In addition, *tif1γ/Tif1γ* is extremely articulated in hematopoietic cells during embryogenesis in both zebrafish and mouse. And as anticipated, forced expression of wild-type *tif1γ* mRNA proficiently releases hematopoiesis in *mon* mutants and does not perturb hematopoiesis in wild-type embryos. We have also cloned the foretold zebrafish ortholog of *tif1α,* which is more consistently expressed in zebrafish embryos like mammalian *TIF1α* and may consequently be accessible to form hetero-oligomers with Tif1γ protein in emergent hematopoietic cells. Comparing existing zebrafish and mammalian TIF1-predicted amino acid sequences, it seems that the Tif1γ orthologs are the most extremely preserved family members while the Tif1α sequences are comparatively more different. We have not also found a Tif1β ortholog, thus far, in the zebrafish or *fugu* genome or EST sequences. It is probable that Tif1β, like the KRAB domain transcription factors it binds to, may be existing only in tetrapods. Nevertheless, more comprehensive genome sequences will be desirable to approve this hypothesis. Based on our examination of zebrafish *mon* mutants, it is realistic to forecast that Tif1γ, the most evolutionarily preserved TIF1 family participant, plays a correspondingly indispensable role in human and mouse hematopoiesis.

Mutations in *tif1γ* cause apoptosis of Erythroid Progenitors

Our inspection of hematopoietic gene expression, apoptosis, and marrow histology in *mon* mutants validates that early erythroid progenitors are made in homozygous mutants, but they fail to appropriately discriminate and in its place undergo programmed cell death. The expression of *gata1* seems to initiate generally in the committed erythroid cells of *mon* mutants. Though, the cells are irregular preceding to the broad loss of *gata1* expression. TUNEL-positive apoptotic cells are copious by the 12-somite phase of development, and by 22 hpf all hematopoietic gene expression is dowsed. The expression of marker genes, comprising of *scl* and *gata2,* characteristic of

hematopoietic stem cells and primitive hematopoietic progenitors, are also not noticed in the embryonic blood islands of mutants at 22 hpf (Zhong et al. 2000). This clearly indicates that the mutant hematopoietic cells are not blocked preceding to the commitment to the erythroid lineage, but in its place develop as abnormal erythroid cells and experience apoptosis, analogous to *gata1*-deficient erythroid cells. Imperfect erythropoiesis and unadorned anemia were also detected in rare surviving homozygous mutant *mon* adults, signifying that *tif1γ* is also essential in definitive hematopoiesis.

The zygotic phenotypes of *mon* mutants might not disclose the purpose of maternally hereditary Tif1γ. Maternally expressed zebrafish Tif1γ might play characters in hematopoiesis or other features of organogenesis that are not obvious due to the existence of wild-type mRNA in eggs laid by heterozygous mothers. Investigation of the offspring of homozygous *mon* mutant female zebrafish will support in describing the purpose of this maternal mRNA. The existing investigation of zygotic *mon* mutants offers data that are consistent with the supposition that *tif1γ* is indispensable for erythropoiesis but do not rule out necessary functions in other hematopoietic lineages.

The hematopoietic phenotype of *mon* mutants look like the loss of erythroid cells perceived in both mouse *Gata1* knockout embryos and zebrafish *vladtepes (gata1)* mutant embryos. In an exertion to determine if there is a hereditary relationship between *mon* and *gata1,* we established their capability to rescue erythropoiesis. Both the mechanism of injection of *gata1* mRNA into *mon*homozygous mutant embryos and the mechanism of injection of *tif1γ* mRNA into *gata1* knock-down embryos was futile to rescue hematopoiesis. We also verified for a direct contact between Tif1γ and Gata1 proteins by co-immunoprecipitation and mold two-hybrid assays and found no connotation. Though the mutations in each of these genes halt cells at a similar stage of development, our outcomes recommend that *gata1* and *tif1γ* act individually. This does not rule out the probability that parallel genetic pathways concerning *gata1* and *tif1γ,* operating together, control gene transcription within blood.

The precise biologic functions of individual miRNAs are now developing through reverse genetic studies, enlightening significant characters in development, physiology and disease, including hematopoiesis. We have to analyze miR142-3p role in zebrafish hematopoiesis, so we have prepared RT-PCR (reverse transcription PCR) to check whether miR142-3p have any action during zebrafish hematopoiesis and when it has high action precisely, so we understood that it has the maximum activity at 24 hpf and 48 hpf (hours post fertilization) and also we have analyzed whether miR-142-3P is mandatory for erythoid formation repression and myeloid expansion in zebrafish through O-dianisidine staining and Whole-mount In Situ Hybridization examination, and our results revealed (with gata1 marker expression) that miR142-3p is mandatory for erythropoiesis because miR142-3p produced reduction for erythroid formation as stated in some previous readings that Gata1 is necessary for erythropoiesis and megakaryocytopoiesis. And (with PU.1 marker expression) we found out that miR142-3p also mandatory for myeloid formation because miR142-3p caused rise in myelopoiesis and also produced migration disorder (Detrich et al. 1995) for myeloid progenitor cells while alternative study prove that, in zebrafish, spi1 (PU1) marks a rostral population of lateral plate mesoderm (LPM) cells dedicated to a myeloid fate anatomically detached from and developmentally autonomous of erythroid commitment in the caudal (LPM).

CONCLUSIONS

All vertebrates, including bony fish, have dual waves of hematopoiesis. The former of these is referred as the primitive or embryonic hematopoietic wave, and predominantly yields erythrocytes, as well as some embryonic macrophages. In zebrafish, primitive hematopoiesis happens in two intraembryonic locations: the intermediate cell mass situated in the trunk ventral to the notochord, and the rostral blood island ascending from the cephalic mesoderm. The zebrafish has appeared as an ideal creature for the study of hematopoiesis, the procedure by which all the cellular elements of the blood are shaped. Large-scale forward genetic screens have recognized plentiful blood mutants in zebrafish, serving to clarify explicit signaling pathways significant for hematopoietic stem cells and the numerous committed blood cell lineages. During development, hematopoietic progenitor cells in mon mutants fail to prompt normal levels of hematopoietic transcription factors including gata1 and undergo apoptosis. Three dissimilar mon mutant alleles individually encode premature stop codons, and imposed expression of wild-type tif1γ mRNA releases embryonic hematopoiesis in homozygous mon mutants. A great level of zygotic tif1γ mRNA expression defines ventral mesoderm during hematopoietic stem cell and progenitor creation prior to gata1 expression. Researches in murine erythroid cell lines validate that Tif1γ protein is restricted within novel nuclear foci, and expression declines during erythroid cell maturation. Our results establish a chief part for this transcriptional intermediary factor in the differentiation of hematopoietic cells in vertebrates. Using the In Situ hybridization technique to exegetics the Zebrafish hematopoiesis has been clearly elucidated in this paper.

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