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miR-142-3p is essential for hematopoiesis and affects cardiac cell fate in zebrafish

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ABSTRACT

MicroRNAs (miRNAs) play a pivotal role during embryonic development and are required for proper organogenesis, including hematopoiesis. Recent studies suggest that, in the early mesoderm, there is an interaction between the hematopoietic and cardiac lineages. However, whether miRNAs can affect other lineages remains unknown. Therefore, we investigated whether hematopoietic miR-142-3p modulated the mesoderm formation. We report that knockdown (KD) of miR-142-3p, a hematopoietic-specific miRNA, in zebrafish resulted in loss of hematopoiesis during embryonic development. Intriguingly, we observed abnormal cardiac phenotypes and insufficiency of somitegenesis in KD-morphants. In the early developmental stage, a tiny heart, contractile dysfunction in the ventricle, cardiac arrhythmia (e.g. a 2:1 ratio of atrial:ventricular beating), and bradycardia were consistently observed. Histological examination revealed severe hypoplasia of the ventricle and disrupted muscle alignment. To determine the mechanism, we performed DNA microarray analysis. The results revealed that the expression of several mesodermal genes essential for the formation of cardiac and somatic mesoderm, such as no tail, T-box gene 16, mesoderm posterior a, one eye pinhead, and rho-associated, coiled-coil containing protein kinase (Rock2a), were increased in miR-142-3p KD-morphants. The luciferase reporter assay revealed that miR-142-3p repressed luciferase activity on the *Rock2a* 3′-UTR. The findings of the present study indicate that miR-142-3p plays a critical role in hematopoiesis, cardiogenesis, and somitegenesis in the early stage of mesoderm formation via regulation of *Rock2a*.

1. Introduction

MicroRNAs (miRNAs) are approximately 22 nucleotides in length and inhibit translation by interacting with the 3′-untranslated regions (3′-UTR) of specific mRNA targets [1]. Moreover, miRNAs have been shown to be involved in modulation of tissue- and cell-specific processes, such as hematopoiesis [2]. Abundant expression of miRNAs may promote differentiation by repressing transcripts that impede cellular commitment [3].

The role of miRNAs in hematopoiesis has been analyzed systematically. Expression analysis in various organs and tissues has shown that miR-181, miR-223, and miR-142 are largely restricted to hematopoietic cells [4]. Although considerable research has been undertaken into the transcriptional regulation of hematopoiesis, more functional studies are required to clarify the mechanisms underlying the pivotal role of individual miRNAs in vivo.

In mammals, primitive hematopoiesis occurs in the yolk sac, later moving to the aorta–gonad–mesonephros (AGM) region and the fetal liver; in contrast, definitive hematopoiesis in adults occurs in the bone marrow [5]. The zebrafish has proven to be a valuable model organism for genetic studies of mammalian hematopoiesis [6]. In zebrafish, hematopoiesis occurs first in the intermediate cell mass (ICM) and subsequently in the AGM region and caudal hematopoietic tissue (CHT). Later, hematopoietic cells are found in the kidney, as well as in the thymus [7]. Indeed, several other miRNAs, such as miR-144 and miR-451, have recently been investigated for their effects on erythroid differentiation in zebrafish [8].

Recent studies have revealed that the hematopoietic and cardiac cell fates from early mesoderm are closely related to each other [9]. A fate-mapping study in zebrafish reported that the induction of vessel and blood lineages determined the borderline for cardiogenesis [10]. In mammals, the cardiac progenitors isolated from crescent-stage mouse embryos were enriched in transcripts that are commonly expressed in other mesodermal lineages, such as cardiac, endothelial, and hematopoietic lineages [11]. In addition, on the basis of microarray data, miR-142 is expressed in embryonic day E 7.75 cardiac progenitors [12]. In

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the present study, we focused on a hematopoietic miRNA, namely miR-142-3p, which is one of the miRNAs detected in the human heart by a sensitive miRNA profiling method (i.e. mRAP-seq [13]) (Drs. H.M and S.T, pers. comm., 2007). We examined the function of miR-142 in zebrafish hematopoiesis and the effect on the cardiac lineage using morpholino knockdown.

2. Materials and methods

2.1. Zebrafish maintenance and morpholino injection

Transgenic (Tg) zebrafish (*Cmlc2*:GFP and *Gata1*:dsRed) were kept under standard laboratory conditions at 28 °C. Morpholino (MO) antisense oligonucleotides were obtained from Gene Tools [dre-miR-142a-3p MO, TCCATAAAGTAGGAAACACTACA; dre-miR-142a-5p MO, AGTAGTGCTTTCTACTTTATG]. Fertilized embryos were injected with the MO at the 1–4-cell stage.

2.2. Immunohistochemistry

Embryos were fixed for 1 h at room temperature in 4% paraformaldehyde, incubated for 1 h in 10%, 20%, and then 30% sucrose/phosphate-buffered saline (PBS), embedded in OCT compound, and sectioned with a cryostat. Sections were stained with hematoxylin–eosin (H&E).

2.3. O-Dianisidine stain

Embryos were dechorionated at 36 h post-fertilization (hpf) and stained for 15 min in 0.6 mg/mL O-dianisidine (D9143; Sigma), sodium acetate (0.01 M, pH 4.5), H_2O_2 (0.65%), and ethanol (40%).

2.4. Expression of miR-142-3p in mouse tissues (real-time quantitative reverse transcription-polymerase chain reaction)

All animal experiments were reviewed and approved by the Institutional Animal Care and Use Committee at the Keio University School of Medicine (#08089). Total RNAs were isolated from different tissues of adult and embryo mice using mirVana (Ambion). The concentration and quality of the isolated RNA were determined spectrophotometrically. After DNaseI (Invitrogen) treatment, 10 ng RNA was reverse-transcribed using the TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems) and with each primer and probe set (hsa-miR-142-3p, 000464; U6 snRNA, 001973). Real-time polymerase chain reaction (PCR) was performed using TaqMan MicroRNA Assays according to the manufacturer's instructions.

2.5. DNA microarray analysis

Zebrafish genome-wide gene expression analysis was performed using the Affymetrix GeneChip zebrafish genome array. RNA was extracted from 24 hpf embryos using an RNeasy mini kit (QIAGEN) according to the manufacturer's instructions. Microarray analysis was performed using the standard protocol supplied with the Affymetrix GeneChip.

2.6. RT-PCR

RNA was prepared from 24 hpf whole embryos injected with MO. For mRNA RT-PCR, cDNA was reverse transcribed with an oligo(dT) primer. Primers for *Danio rerio* β-actin, the embryonic α-, β-globin genes (*Hbae1* and *Hbba1*, respectively), adult α-globin gene (*Hbaa1*), myogenic differentiation 1 (*MyoD*), myogenic factor 5 (*Myf5*), no tail (*Ntl*), bHLH transcription factor mesp-a (*Mespa*), one-eyed pinhead (*Oep*), T-box gene 16 (*Tbx16*), T-cell acute lymphocytic leukemia 1 (*Scl/Tal1*), bone morphogenetic protein 2b (*Bmp2b*), Spleen focus forming virus proviral integration oncogene spi1 (*Pu.1*), GATA binding protein 1a (*Gata1*), GATA-binding protein 2a (*Gata2*), and rho-associated, coiled-coil containing protein kinase (*Rock2a*) were designed as indicated in Table S1, available as Supplementary material for this paper.

2.7. In situ hybridization

In situ hybridization using locked nucleic acid probes against miR-142-3p (Exiqon) was performed as described previously [14].

2.8. Luciferase assay

For the luciferase assay, 1205 nucleotides (4226–5430; NM_174863) of the *D. rerio* rock2a 3′-UTR, predicted to contain miR-142a-3p-binding sites, were cloned into the pMIR-REPORT miRNA expression reporter vector (Ambion). Pri-miR-142a and pri-miR-126 were subcloned into the pcDNA3.1 vector (Invitrogen). Renilla-encoding vector was used as a transfection control. COS7 cells were transfected with 100 or 300 ng pcDNA3.1/dre-miR-142a or pcDNA3.1/dre-miR-126, 1 μg pMIR/rock2a 3′-UTR, and 10 ng Renilla constructs with FuGene (Roche) and were harvested after 24 h. Luciferase and Renilla activity was assayed using the Dual-Reporter Assay (Promega). Data shown are from experiments performed in triplicate.

2.9. Statistical analysis

All experiments were performed at least three times. Data are given as the mean ± SD. Student's *t*-test was used for statistical comparisons. $P < 0.05$ was considered significant.

3. Results

3.1. miR-142-3p is highly enriched in all hematopoietic tissues

The mature sequence of miR-142 found in several species, including human, mouse, and zebrafish, is highly conserved (Supplemental Fig. 1A). The pre-miR-142 stem loop has two mature miRNAs, namely miR-142-3p and miR-142-5p, in a hairpin structure (Supplemental Fig. 1B). Northern blot analysis to characterize miRNA localization in mouse tissues revealed that miR-142s were highly expressed in all hematopoietic tissues [4]. In the present study, to determine whether miR-142-3p was enriched in hematopoietic tissues in vivo, we used quantitative (q) RT-PCR with RNA from tissues obtained from adult and embryonic mice. We found that miR-142-3p was highly expressed in all hematopoietic tissues, including the bone marrow, spleen, thymus, and fetal liver. High expression on E12.5 in the fetal liver, an embryonic hematopoietic system, implies that miR-142-3p may play a pivotal role in early hematopoietic development (Supplemental Fig. 2A, B). In zebrafish, miR-142-3p expression was induced in 24 hpf embryos and increased between 48 and 72 hpf (Fig. 1A).

To examine the anatomical localization of miR-142-3p, we determined the miR-142-3p expression profile in zebrafish embryos using whole-mount in situ hybridization (WISH). At 30 hpf, miR-142-3p was detected in erythrocytes pooling over the yolk during fixation for WISH (Fig. 1B).

3.2. miR-142-3p modulates somitogenesis in vivo

The mature miRNA sequences of zebrafish miR-142-3p and miR-142-5p match those of their mammalian orthologs. Because

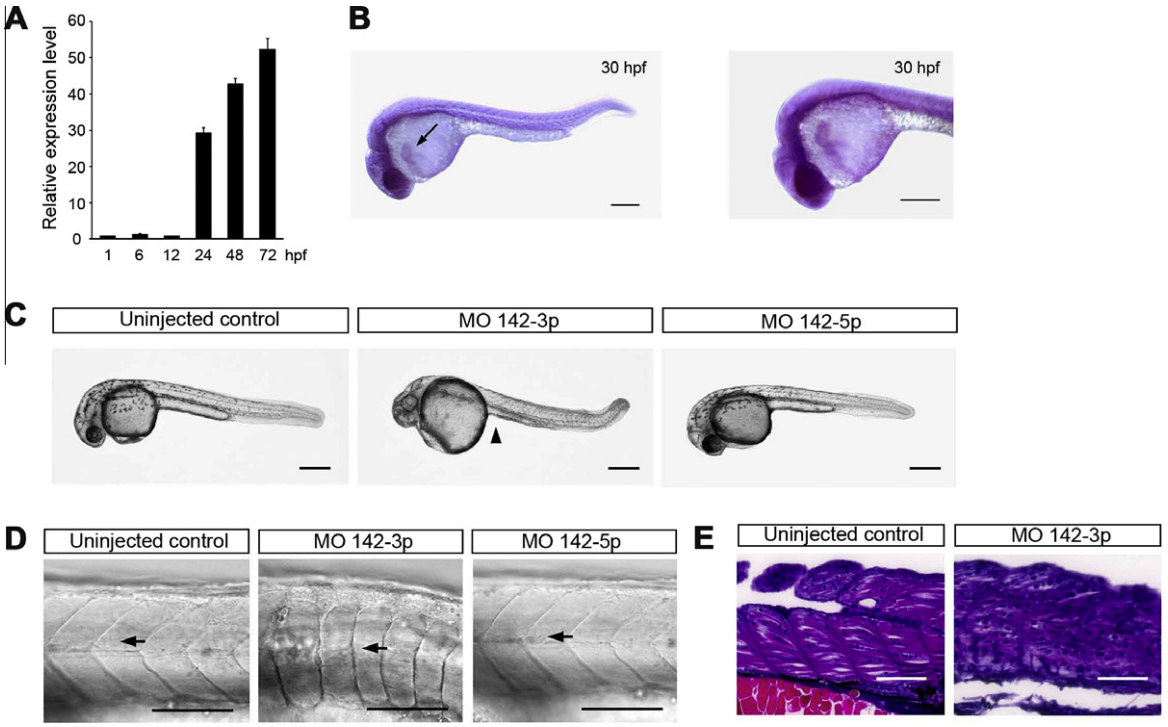

Fig. 1. MiR-142-3p is highly expressed in the yolk and inhibition of miR-142-3p results in disrupted somitogenesis. (A) Quantification of miR-142-3p at different embryonic stages by qRT-PCR. (B) Whole mount in situ hybridization (WISH) analysis of miR-142-3p at 30 h post fertilization (hpf). Expression of miR-142-3p was detected over the yolk (arrow). Lateral views, anterior to the left. Original magnification ×5 (left panel) and ×8 (right panel). Scale bar, 300 μm. (C) Lateral views (anterior to the left) are shown of uninjected control, miR-142-3p morpholino-injected zebrafish ($MO^{142\text{-}3p}$) and miR-142-3p morpholino-injected zebrafish ($MO^{142\text{-}5p}$) at 30 hpf. Bright field microscopy revealed decreased melanogenesis and inadequate yolk extension (arrowhead). Scale bar, 300 μm. (D) Lateral views of the trunk region of uninjected control, $MO^{142\text{-}3p}$ and $MO^{142\text{-}5p}$ embryos at 30 hpf. The somite boundary was irregular in $MO^{142\text{-}3p}$ embryos. Scale bar, 100 μm. (E) H&E-stained sections of the trunk at 72 hpf. Alignment of muscle filaments is irregular in $MO^{142\text{-}3p}$ embryos. Scale bars, 100 μm.

temporal inhibition of miRNAs by antisense molecules is the approach used to analyze the loss of function of miRNA, we used it in the present study in zebrafish embryos. Microinjection into fertilized eggs of an antisense MO targeting miR-142-3p decreased miR-142-3p function during zebrafish development.

At 6 hpf, MO-injected embryos did not exhibit any differences compared with uninjected embryos. However, at 30 hpf, gross morphological examination revealed that pigmentation of the skin appeared obscurely and yolk extension was inadequate (Fig. 1C, arrowhead) in miR-142-3p MO-injected zebrafish ($MO^{142\text{-}3p}$). Closer examination revealed that somite formation was weak globally (Fig. 1D). To evaluate the defects in the MO-injected embryos further, we examined histological sections. In wild-type embryos, regular alignment of muscle filaments is clearly seen. In contrast, in longitudinal H&E-stained sections from $MO^{142\text{-}3p}$ at 72 hpf, bundles of muscle filaments were disrupted (Fig. 1E). We verified the specificity of the $MO^{142\text{-}3p}$-induced phenotypes by injection of miR-142-5p MO ($MO^{142\text{-}5p}$). The gross phenotypes seen in $MO^{142\text{-}3p}$ embryos were not observed in $MO^{142\text{-}5p}$ embryos (Supplemental Fig. 3).

3.3. miR-142-3p is essential for hematopoiesis in vivo

The presence of circulating blood cells was greatly reduced at 48 hpf in $MO^{142\text{-}3p}$ (Fig. 2A, B). We analyzed the percentage of gross phenocopies of hematopoietic defects at 30 hpf. Approximately 92% ($n = 102/111$) of $MO^{142\text{-}3p}$ embryos exhibited markedly decreased circulating erythrocytes. We confirmed the effect of inhibition of miR-142-3p by using Tg *Gata1*:dsRed reporter zebrafish, which express the transgene in blood cells. Although circulation of blood occurred normally between 24 and 30 hpf, the presence of Tg gata1:dsRed-expressing blood cells in vessels was markedly reduced in $MO^{142\text{-}3p}$ embryos (Fig. 2C and Supplemental Fig. 4).

In non-injected and $MO^{142\text{-}5p}$ embryos at 36 hpf, the appearance of erythroid cells in the vessels was evidenced by O-dianisidine staining. In contrast, $MO^{142\text{-}3p}$ embryos caused severe defect of erythrocytes indicated by reduced hemoglobinized cells throughout the embryo (Fig. 2D).

We injected 0.2 mM and 1.0 mM morpholino, and the percentage of the reduction of erythroid cells was 29% ($n = 7/24$), 94% ($n = 49/52$), respectively. These data imply that MO dose-dependently impairs hematopoiesis in zebrafish embryos (Fig. 2E).

3.4. miR-142-3p has an effect on cardiac function

Inhibition of miR-142-3p resulted in cardiac malformations. By using a Tg *Cmlc2*:GFP reporter zebrafish, which expresses green fluorescent protein (GFP) in cardiomyocytes, we assessed the effect of miR-142-3p knockdown on cardiac development.

The $MO^{142\text{-}3p}$-injected phenotype was first recognized at around 24 hpf as a tiny heart and bradycardia compared with wild-type embryos, in which the heart has completed tube formation and starts beating at this stage. At 48–72 hpf, embryos with the cardiac phenotype showed a looping abnormality and contractile dysfunction of the ventricle (Fig. 3A, D). Histological examination revealed that the ventricular wall appeared thinner in these embryos than in wild-type embryos (Fig. 3B). However, the $MO^{142\text{-}5p}$-injected phenotype showed very few cardiac anomalies (Fig. 3C). Furthermore, $MO^{142\text{-}3p}$-injected embryos exhibited cardiac arrhythmias, such as a 2:1 ratio of atrial:ventricular beating, and pericardial edema (Fig. 3D). The average heart rate of morphants was significantly slower than that of wild-type embryos (Fig. 3E). To quantify

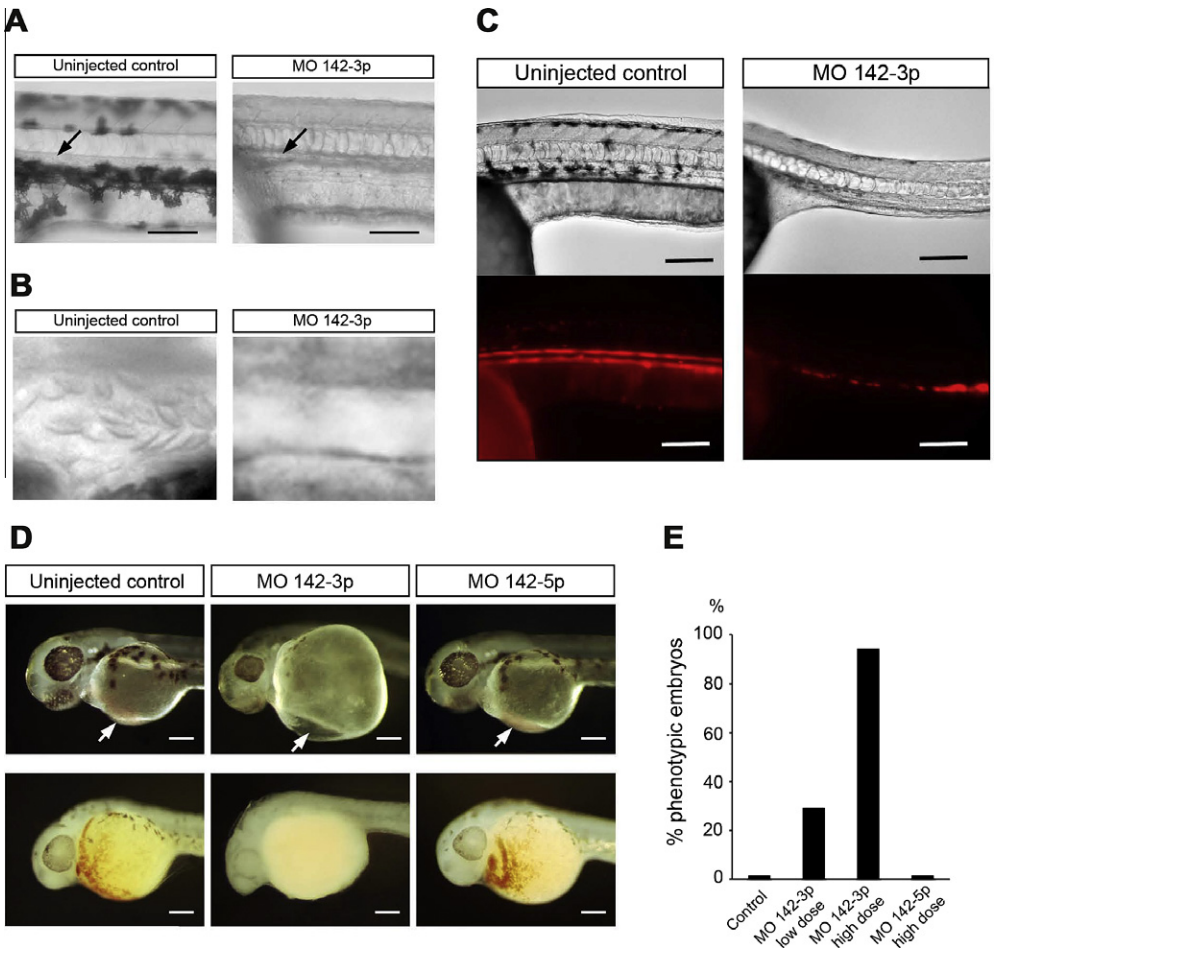

Fig. 2. miR-142-3p is required for the maturation of erythrocytes. (A) Inhibition of miR-142-3p resulted in a reduction of blood cells in vessels. Bright field images (48 hpf). Scale bar, 300 μm. (B) Higher magnification view of the blood vessels revealing a reduction of blood cells. (C) Lateral views of wild-type and miR-142-3p morpholino-injected transgenic (Tg; *Gata1*:dsRed) zebrafish at 30 hpf. There was a significant reduction in blood cells (*Gata1*:dsRed) in the vessels in the morphants. Scale bar, 300 μm. (D) Inhibition of miR-142-5p does not cause hematopoietic defects. There is a significant reduction of blood cells in vessels in $MO^{142\text{-}3p}$ embryos. Decreased hemoglobin staining by O-dianisidine was evident at 36 hpf. Normal hemoglobin expression was seen in wild-type (WT) and $MO^{142\text{-}5p}$ embryos. Scale bar, 300 μm. (E) There were a greater number of $MO^{142\text{-}3p}$ embryos with decreased circulating cells at 36 hpf.

cardiac function, end-diastolic and end-systolic areas of wild-type and morphant ventricles were measured and the percentage fractional area change (%FAC) calculated. At 60 hpf, a decrease in %FAC was observed in $MO^{142\text{-}3p}$-injected embryos (Fig. 3F). Together, these results demonstrate that miR-142-3p modulates normal cardiac formation and function.

3.5. Identification of genes affected by miR-142-3p inhibition

To determine the mechanisms underlying the effects of miR-142-3p, we used DNA microarray to analyze the mRNA expression pattern in whole embryos injected with $MO^{142\text{-}3p}$ and compared it with the pattern in non-injected embryos or those injected with $MO^{142\text{-}5p}$. In the present study, we defined $MO^{142\text{-}3p}$-upregulated genes as follows: (i) the ratio of expression between $MO^{142\text{-}3p}$ and $MO^{142\text{-}5p}$ embryos was $\geqslant 1.7$; (ii) the ratio of expression between $MO^{142\text{-}3p}$ and wild-type embryos was $\geqslant 1.7$; and (iii) the expression in $MO^{142\text{-}3p}$ embryos was within the upper 30%. The $MO^{142\text{-}3p}$-downregulated genes were defined as those for which: (i) the ratio of expression in $MO^{142\text{-}3p}/MO^{142\text{-}5p}$ was $\leqslant 0.2$; (ii) the ratio of expression in $MO^{142\text{-}3p}$/wild-type was $\leqslant 0.2$; and (iii) expression in $MO^{142\text{-}5p}$ or wild-type embryos was within the upper 20%. Furthermore, we excluded genes that have not been described in detail (e.g. those with only "transcribed sequences") and created a list of up- and down-regulated genes (see Supplemental Tables 2 and 3, respectively).

Several mesodermal genes were upregulated, including *Ntl*, *Tbx16*, *Mespa*, and *Oep*. Numerous genes that have established roles within the hematopoietic stem cell compartment or in red cell precursors were also upregulated in $MO^{142\text{-}3p}$, including *Scl/Tal1*, *Bmp2b*, and *Gata2*. *Gata1*, a gene required for the terminal differentiation of erythroid cells, was increased slightly in $MO^{142\text{-}3p}$ embryos. *Pu.1*, a master regulator of myeloid cell development, was also increased. Conversely, marked downregulation was observed in the expression of *Hbae1*, *Hbba1* and *Hbaa1*.

Upregulated expression of premature muscle-related genes, such as *MyoD* and *Myf5*, was also observed, and there was marked downregulation of the expression of *Cmlc*, *α-actin*, and several troponin types. However, microarray analysis did not detect any significant changes in the expression of NK2 transcription factor related 5 (*Nkx2.5*) or heart and neural crest derivatives expressed transcript 2 (*Hand2*), both of which are key regulators in cardiac progenitor cells.

The expression patterns revealed by microarray analysis were verified by RT-PCR (Fig. 4A). These results implied that not only is miR-142-3p crucial for the maturation of hematopoietic cells, but that it also affects cardiac and muscular cell fate from the mesoderm at the early stage of development.

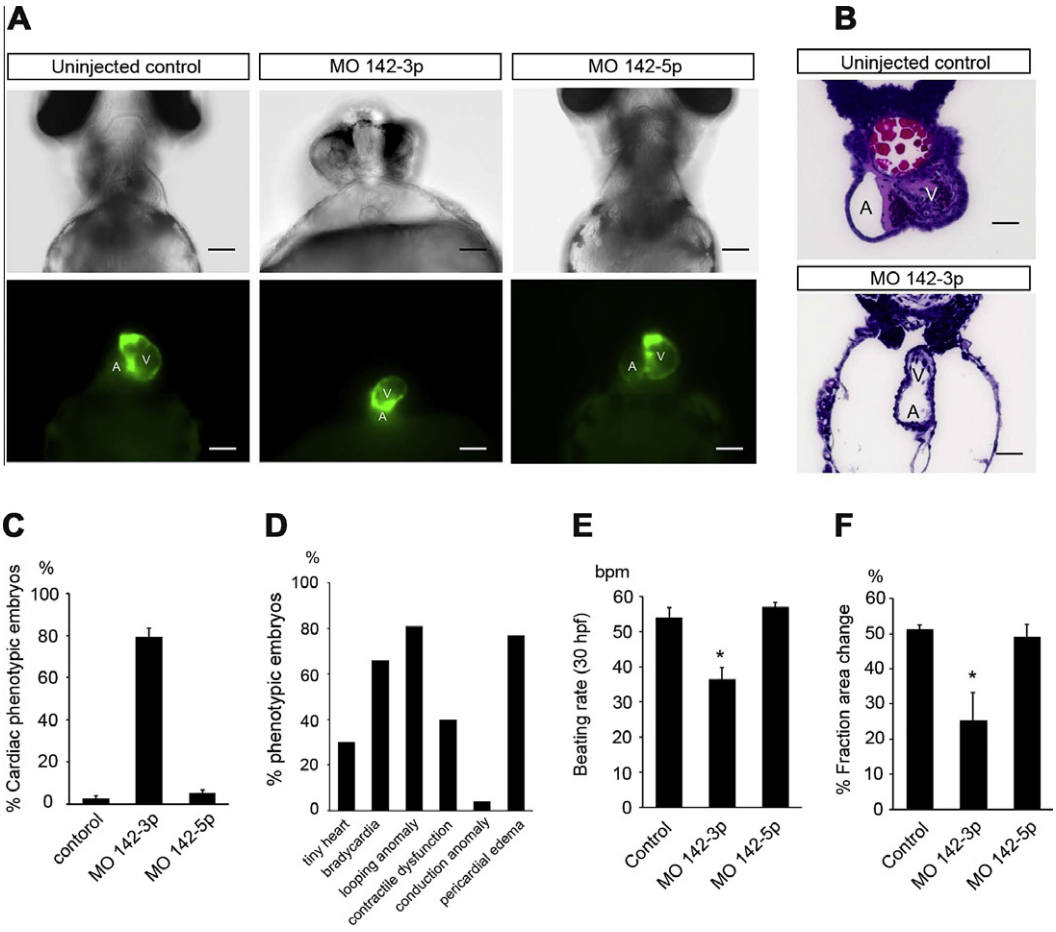

Fig. 3. Inhibition of miR-142-3p results in cardiac abnormalities and depressed cardiac function. (A) Ventral view of the heart in wild-type (WT), miR-142-3p morpholino (MO)-injected transgenic (Tg; *Cmlc2*:GFP) zebrafish and miR-142-5p MO-injected transgenic (Tg; *Cmlc2*:GFP) zebrafish at 60 hpf. Bright field microscopy (upper) and fluoroscopy (lower) demonstrated looping anomalies, small cardiac chambers, and pericardial edema in $MO^{142\text{-}3p}$ embryos. Scale bar, 100 μm. A, atrium; V, ventricle. (B) Transverse H&E-stained sections of the heart showing a thinner ventricular wall in morphant embryos at 72 hpf (bottom). Scale bar, 100 μm (C) The percentage of wild-type, $MO^{142\text{-}3p}$, and $MO^{142\text{-}5p}$ embryos with cardiac abnormalities. (D) The percentage of $MO^{142\text{-}3p}$ embryos exhibiting different cardiac phenotypes. (E) The average heart rate of $MO^{142\text{-}3p}$ embryos was lower than that of wild-type and $MO^{142\text{-}5p}$ embryos at 30 hpf ($n = 5$). $^*P < 0.05$. (F) Contractility as percentage fractional area change (%FAC) in wild-type, $MO^{142\text{-}3p}$, and $MO^{142\text{-}5p}$ hearts at 60 hpf ($n = 3$). $^*P < 0.05$.

3.6. miR-142-3p represses rock2a in the luciferase reporter assay

To elucidate the mechanism by which miR-142-3p regulates mesodermal differentiation, we searched for potential direct mRNA targets of miR-142-3p. Several mRNA targets were selected from the TargetScan (http://www.targetscan.org/index.html) databases. We compared potential targets for the presence of an miR-142-3p-binding site conserved in the 3′-UTR in zebrafish. From this analysis, a conserved miR-142-3p-binding site was identified in the *Rock2* 3′-UTR. There are two potential binding sites that match positions 2–7 of the mature miRNA (the seed; Fig. 4B).

Both Rock1 and *Rock2* play important roles in cell proliferation and apoptosis. In zebrafish, *Rock2* proteins are endogenous inhibitors for mesoderm induction [15]. In the present study, we cloned the partial length of the 3′-UTR of the zebrafish *Rock2a* sequence into the luciferase reporter. The 3′-UTR sequence inhibited luciferase activity in response to miR-142, but not miR-126, for which there was no predicted binding site (Fig. 4C).

4. Discussion

We used reverse genetic techniques in the zebrafish to determine the function of miR-142-3p and demonstrated that miR-142-3p regulates hematopoiesis, cardiogenesis, and somitogenesis in vivo. Expression analysis revealed that genes such as *Scl/Tal1* and *Gata2* were upregulated in $MO^{142\text{-}3p}$ embryos. *Scl* is a basic helix-loop-helix transcription factor and is expressed from the 2- to 3-somite stage in the hemangioblast population, together with *Gata2* [16]. Knockdown of *Scl* in zebrafish resulted in the disturbance of primitive erythropoiesis and myelopoiesis. *Gata2* is a zinc finger transcription factor that is required for the proliferation and maintenance of hematopoietic progenitor cells [17]. In mammalian hematopoiesis, persistent production of GATA2 maintains a stem cell phenotype and reductions in *Gata2* expression or activity are required for the differentiation of precursors to hematopoietic cells [18]. Moreover, gata1-positive cells were markedly reduced in $MO^{142\text{-}3p}$ embryos. *Gata1* is a master transcription factor in erythrocyte maturation. In zebrafish, *Gata1* is expressed from the 5-somite stage in the posterior lateral mesoderm along with *Scl* and *Gata2*. From the 12-somite stage, *Gata1*-positive cells of the ICM migrate anteriorly and then start to express erythroid-specific genes and circulate. [19]. During erythroid differentiation, both GATA1 and GATA2 nucleate the binding of various protein complexes containing SCL following globin gene activation [20]. The persistent expression of *Gata2* and *Scl* following knockdown of miR-142-3p may have impaired intact differentiation. On the basis of these findings, we think that miR-142-3p may affect the

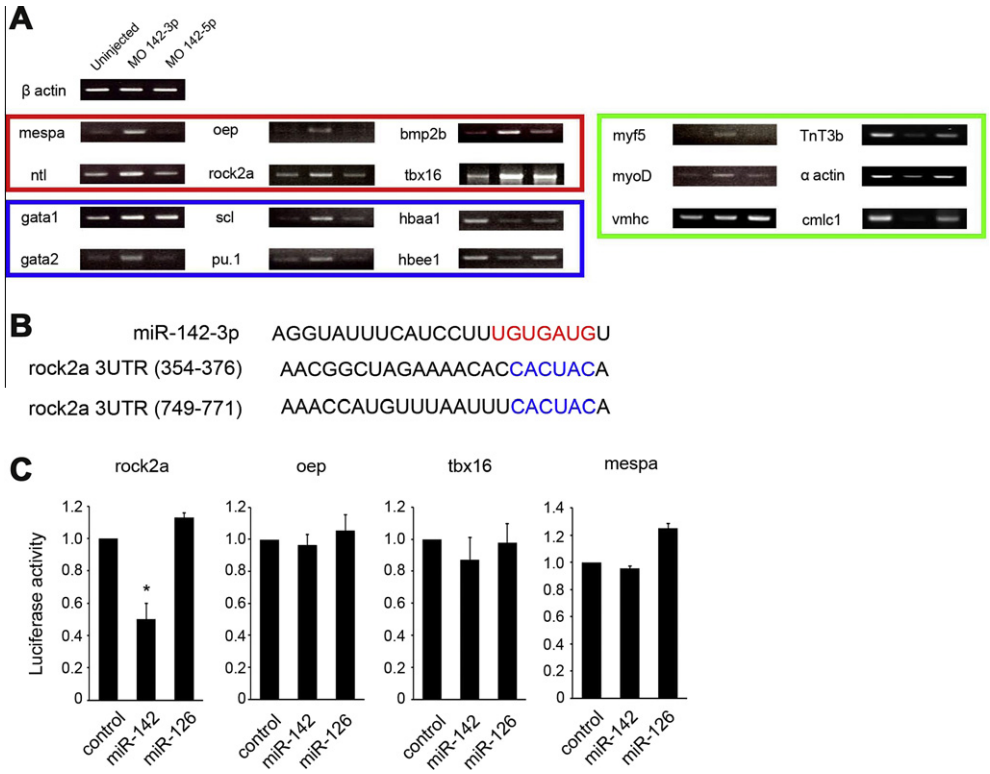

Fig. 4. Identification of miR-142-3p targets. (A) Gene expression, as determined by reverse-transcription polymerase chain reaction (RT-PCR), at 24 hpf. (B) Sequence complementarities of a potential miR-142-3p-binding site in the rho-associated, coiled-coil containing protein kinase (*Rock2a*) 3′-untranslated region (3′-UTR). (C) Relative luciferase activity of constructs containing the 3′-UTR of the potential miR-142-3p binding site transfected into COS7 cells in the presence of miR-142 or miR-126 ($n = 3$). $^{*}P < 0.05$.

conversion from hematopoietic progenitor cells to mature erythrocytes that possess abundant hemoglobin.

In the present study, miR-142-3p was highly expressed in all hematopoietic tissues, but not in other tissues, including the heart. However, knockdown of miR-142-3p affected cardiac and muscular development. According to the microarray data, expression of *Tbx16*/*Spadetail*, a T-box transcription factor involved in mesoderm development, was increased in $MO^{142\text{-}3p}$ embryos. The mutant phenotype of *Spadetail* shows disrupted primitive erythrocytes, as well as disrupted somitogenesis. In addition, *Spadetail* is required for cardiac mesoderm formation via interaction with the Nodal-signaling pathway as *Oep*. Hence, a possible explanation for the cardiac and somatic phenotypes may be the persistent expression of transcriptional factors that, under normal conditions, appear only transiently in the early mesoderm. MiR-142-3p may affect genes that play a key role in the early stage of mesoderm formation, resulting in disrupted hematopoiesis, cardiogenesis, and somitogenesis in $MO^{142\text{-}3p}$ embryos. As a result, the expression of genes in each mature lineage could have been decreased.

Recently, the interaction between hemangioblasts and cardiac progenitors in the anterior lateral plate mesoderm (ALPM) has attracted attention. Induction of the hematopoietic fate represses cardiac specification and delimits the capacity of the heart field. In addition, scl is essential for overriding the latent cardiac developmental potential residing within the rostral ALPM and thereby limiting heart size. [10]. In contrast, cardiac precursors can antagonize the hematopoietic lineage in embryonic stem cells. Overexpression of *Nkx2.5* represses hematopoietic differentiation. It has been proposed that *Nkx2.5* has a dual role in multipotent mesodermal progenitors of promoting a cardiac fate and repressing a hematopoietic fate [21].

Mammalian early cardiac progenitors are known to express hematopoietic genes. The cardiac progenitors extracted from crescent-stage embryos have abundant hematopoietic genes [11]. In addition, miR-142-3p is observed in E7.75 cardiac progenitors, as determined on the basis of microarray data [12]. Based on this early expression pattern, we hypothesize that miR-142-3p is required in the early mesoderm and is a key regulator of the interaction between both blood- and cardiac-inducing signals. Although it remains contentious as to whether a common progenitor for cardiac and hematopoietic lineages exists, mesodermal cells possibly have multilineage potential and can differentiate into hematopoietic and cardiac cells depending on the environment. As a future investigation, it would be interesting to clarify how miRNAs, not just mRNAs, regulate cell fate determination between hematopoietic and cardiac lineages from multipotent progenitors in mesoderm formation.

Using the luciferase assay, we showed that one of the possible targets of miR-142-3p is *Rock2*, which phosphorylates a variety of substrates, including myosin light chain. In addition, Rho kinases, effectors of Rho small GTPase, have been found to be important regulators of cell contraction, adhesion, and migration, as well as proliferation and apoptosis. Several reports indicate that Rho GTPase signaling has essential roles in blood cell development and function [22], as well as in the development of an intact mesoderm, including cardiogenesis [23]. The fine-tuning effect of miR-142-3p in the early mesoderm may involve modulation of the Rho GTPase signals to regulate specific steps of erythropoiesis and cardiogenesis via *Rock2*.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.07.148.

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