Current Molecular Medicine 2017, *17*, 627-636

RESEARCH ARTICLE

Role of Zebrafish *fhl1A* in Satellite Cell and Skeletal Muscle Development

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Abstract: ***Background*:** Four-and-a-half LIM domains protein 1 (FHL1) mutations are associated with human myopathies. However, the function of this protein in skeletal development remains unclear.

***Methods*:** Whole-mount in situ hybridization and embryo immunostaining were performed.

***Results*:** Zebrafish Fhl1A is the homologue of human FHL1. We showed that *fhl1A* knockdown causes defective skeletal muscle development, while injection with *fhl1A* mRNA largely recovered the muscle development in these *fhl1A* morphants. We also demonstrated that *fhl1A* knockdown decreases the number of satellite cells. This decrease in satellite cells and the emergence of skeletal muscle abnormalities were associated with alterations in the gene expression of *myoD*, *pax7*, *mef2ca* and *skMLCK*. We also demonstrated that *fhl1A* expression and retinoic acid (RA) signalling caused similar skeletal muscle development phenotypes. Moreover, when treated with exogenous RA, endogenous *fhl1A* expression in skeletal muscles was robust. When treated with DEAB, an RA signalling inhibitor which inhibits the activity of retinaldehyde dehydrogenase, *fhl1A* was downregulated.

***Conclusion*:** *fhl1A* functions as an activator in regulating the number of satellite cells and in skeletal muscle development. The role of *fhl1A* in skeletal myogenesis is regulated by RA signaling.

ARTICLE HISTORY

Received: January 27, 2018
Revised: February 23, 2018
Accepted: February 24, 2018

DOI:
10.2174/1566524018666180308113909

Keywords: *fhl1A*, satellite cells, skeletal muscle, development, zebrafish, myogenesis.

1. INTRODUCTION

Four-and-a-half LIM domain protein 1 (FHL1) is highly expressed in skeletal muscles, suggesting that the *FHL1* gene is associated with the development of human muscle diseases. Indeed, *FHL1* mutations have been identified in a number of human myopathies [1-3]. As all clinical subtypes with mutations in exons of *FHL1* are associated with myofibril changes and diminished bodies, these muscular diseases may be investigated as resulting from these mutations [1, 2]. *FHL1*-related muscle diseases can be divided into two broad categories: 1) missense mutations in the LIM2 domain affecting the zinc-coordinating histidine and cysteine, and 2) missense mutations in the C-terminal domain-encoding region of FHL1 isoforms [4-10]. In mice, the LIM-only protein FHL1 is primarily expressed in skeletal and cardiac muscles [11, 12]. Overexpression of this protein enhances myoblast fusion, resulting in hypertrophic myotubes in C2C12 cells, as well as modulating muscle mass and enhancing strength via the regulation of NFATc1 signalling [13]. The transgenic expression of FHL1 protein promotes hypertrophy in mouse skeletal muscle and performs various functions in muscle cell lines [14, 15]. It is also reported that the loss of *FHL1* function causes age-dependent myopathy [16]. Although the role of *FHL1* in the development of human myopathies is becoming better understood, the pathological and molecular

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1875-5666/17 $58.00+.00

mechanisms of *FHL1* in skeletal muscle development and disease have yet to be clarified.

Recently, zebrafish have been identified as a useful model of certain human diseases, including myopathies [17]. In zebrafish, axial skeletal muscles are made up of four types of cells: slow-twitch muscle cells, muscle pioneer cells, fast-twitch muscle cells and medial fast fibre cells [18]. These cells, marked by the expression of transcription factors such as *myoD*, initially form from the somites that are patterned into myotomes and sclerotomes [19]. The progenitors of slow-twitch muscle cells, including muscle pioneers, are located on the lateral surfaces of the somites, whereas the progenitors of fast-twitch muscle cells remain deeper within the somites [20]. In addition, the satellite cells, marked by their expression of transcription factors like *Pax7*, are also progenitor cells and can generate the myoblasts required for muscle growth, repair and regeneration [21]. By 28 hpf, slow-twitch and fast-twitch muscle differentiation are well underway. Muscle development requires co-ordinated expression of a family of basic helix-loop-helix (bHLH) myogenic regulatory factors (MRFs): *myoD*, *myogenin*, *myf5* and *MRF4* [22-24]. MyoD directly activates the expression of the other transcription factors, such as *myogenin*, and plays a central role in the induction of myogenic differentiation [25]. Myocyte enhancer factor 2C (*MEF2C*) is expressed at all stages of satellite cell myogenesis and is required for the development of skeletal muscle [26]. A skeletal muscle myosin light chain kinase (*skMLCK*) is important for the activation of satellite cells and their subsequent differentiation [27], as well as for skeletal muscle development. Seale *et al*. showed that deletion of *Pax7* in mice compromises muscle regeneration due to increased satellite cell apoptosis [28]. Moreover, a number of signalling pathways are involved in the process of muscle development: Wnt signalling modulates both the number of terminally differentiated myogenic cells and the intricate slow/fast patterning of the limb musculature [29], while TGF-beta inhibits myogenic differentiation in myoblasts [30]. At present, the molecular mechanism that modulates skeletal muscle development is not fully understood.

Retinoic Acid (RA) has been demonstrated to regulate heart chamber development and promote myogenic differentiation [31-34]. Our previous study revealed that RA signalling restricts heart chamber formation via *fhl1A*, and that *fhl1A* is expressed in the early stages of skeletal muscle and cardiac myocyte development. We, therefore, hypothesised that RA signalling may also affect skeletal muscle development through *fhl1A*. In the present study, we observed the role of zebrafish *fhl1A*, the homologue of human *FHL1*, on skeletal muscle development. We demonstrated that *fhl1A* functions as an activator in regulating the number of satellite cells and in skeletal muscle development.

2. MATERIALS AND METHODS

2.1. Zebrafish Maintenance

Wild-type AB strain zebrafish was raised under standard laboratory conditions at 28.5°C in non-buffered E3.

2.2. Whole-Mount *In Situ* Hybridization

In situ RNA hybridization was performed using digoxigenin-labelled antisense RNA probes. Total RNA was extracted as previously described, then reverse transcribed to cDNA [34]. To construct probes, 400–1000 bp exon sequences of antisense probes for *fhl1A*, *myoD*, *skMLCK* and *mef2ca* were amplified from cDNA using PCR and cloned into pGEM-T or pGEM-T easy vectors (Promega, Madison, WI, USA) [35-37]. They were then transcribed using RNA transcription reagents (Promega). The primer sequence used was as follows: *skMLCK* S: 5′-GCTACAACCCTCCTAAACT-3′; R: 5′-CACTCATACGACCACTTCTT-3′.

2.3. DEAB and RA Treatments

Zebrafish embryos were treated with all-trans RA (Sigma, St. Louis, MO, USA) and DEAB (Sigma) beginning at 40% epiboly as previously described [34]. The embryos were washed in PBST (1X PBS, 0.1% Tween 20) and fixed overnight at the indicated stages at 4°C in 4% paraformaldehyde.

2.4. Whole-Mount Antibody Staining

Embryos at the desired developmental stages were gathered and processed as previously described. Whole-mount embryo immunostaining was performed following standard protocols [38]. The primary antibodies used were as follows: MF20 (1:200 dilutions, DSHB) and Pax7 (1:200 dilutions, DSHB). The fluorescent secondary anti-mouse antibody used for detection was Alex-594-conjugated anti-mouse IgG (Invitrogen, Carlsbad, CA, USA). Stained embryos were photographed using fluorescence optics from Nikon (Nikon, Japan).

2.5. Antisense Morpholinos, Rescue and Overexpression Experiments

Two non-overlapping antisense morpholino oligonucleotides of *fhl1A*-MO1 and *fhl1A*-MO2 targeting the translational start site of zebrafish *fhl1A* (*fhl1A* MO) were synthesised at Gene-Tools (OR, USA), along with standard control morpholinos. The *fhl1A* MO was used as previously described [37]. The *fhl1A*-capped mRNA transcripts of *fhl1A* were synthesised and used as previously described [37]. After injection, embryos were incubated at 28.5°C in embryo medium.

2.6. Statistical Analysis

All data were expressed as mean ± standard deviation using SigmaPlot 11.0. Statistical differences

between the control group and MO-treated groups were determined using the rank sum test. For phenotype classification in the later developmental stages (after 24 hpf), we observed the overall phenotype; *fhl1A* MO-injected embryos with short trunks, abnormal dorsal curved body shape and embryos with pigment loss were defined as defective embryos. Embryos without these phenotypic traits were classified as normal. For embryos in the early developmental stages (before 24 hpf), we carefully checked the somite shape and the body axis morphology, and those with shorter, round somites were defined as defective embryos. Those with longer, narrow somites were defined as normal. Results were considered statistically significant if $p < 0.05$.

2.7. Western Blotting

Wild-type AB strain embryos were treated as described above with DEAB and RA. Zebrafish embryos were manually dechorionated and deyolked before homogenisation. Deyolked samples were dissolved in 2 μL of 2X sodium dodecyl sulphate (SDS) sample buffer per embryo and incubated for 5 min at 95°C. After full-speed centrifugation for 1 min in a microcentrifuge to remove insoluble particles, samples were separated on a 10% SDS polyacrylamide gel. Western blot was performed according to the previous report using the human anti-FHL1 antibody (1:1500 dilution, Proteintech) and anti-β-Actin antibody (1:4000 dilution, Proteintech) [34].

2.8. RT-PCR

PCR amplification was performed in a 25 μL reaction mixture containing 1 μM *pax7a* primers (F: 5′-CCAGAACTACCCACGAAC-3′ and R: 5′-CTTCCACCAATAGCACCC-3′), *pax7b* primers (F: 5′-AAGACGACGAGGATGATTGT-3′ and R: 5′-TCTGGTGTAGATGTCAGGGTAG-3′), *skMLCK* primers (F: 5′-GCTGTCGGAGACCGTAAA-3′ and R: 5′-AATCAGCAGCCAGTCAAA-3′) or *β-actin* primers (F: 5′-TATTGTGATGGACTCTGGTGATG-3′ and R: 5′-TCGGCTGTGGTGGTGAAG-3′), 1.5 mM MgCl, 0.2 mM dNTP, and 1 U Taq (Takara, Japan). The samples were incubated in a thermal cycler (Hybaid MultiBlock System) at 95°C for 5 min; then subjected to 30 cycles of 95°C for 30 s, 55°C for 30 s and 72°C for 30 s; followed by final extension at 72°C for 8 min. All PCR products were separated using electrophoresis on a 1.2% agarose gel.

3. RESULTS

3.1. Expression of *fhl1A* and its Role in Skeletal Muscle Development

We cloned the ORF of *fhl1A* and synthesised a DIG-labelled *fhl1A* antisense RNA probe as previously described [37]. The sense RNA probe was used as a negative control. The expression pattern of zebrafish *fhl1A* during embryogenesis was analysed by whole-mount *in situ* hybridization. As shown in Fig. **1A-C**, *fhl1A* expression was not detected in somites at the 3-somite stage but was detected in somites at the 10- and 20-somite stages, implying that it may not be involved in progenitor skeletal muscle initiation but may play a role in skeletal muscle development. To assess the functions of *fhl1A*, we performed loss-of-function experiments in zebrafish using *fhl1A* MO, which blocks the expression of Fhl1A protein in zebrafish embryos [37]. As shown in Fig. **1E** and **1E'**, the morphant embryos injected with 2 ng *fhl1A*-MO exhibited a defective phenotype, with shorter somites at the 12-somite stage, compared with control embryos injected with 2 ng Control-MO (Fig. **1D** and **D'**). When *fhl1A* MO and *fhl1A*-capped mRNA were co-injected, 89% of the embryos were restored to normal phenotypes (Fig. **1F**, **F'** and **1G**). Stronger defects were observed at 24 hpf; instead of the healthy chevron shape observed in control somites (Fig. **1H**, **1H'**), 67% of the embryos showed slash-shaped somites (Fig. **1I**, **1K**). These somites became shorter and rounder, with broadened somitic furrows throughout the trunk (Fig. **1I'**). When embryos were co-injected with *fhl1A* MO and *fhl1A*-capped mRNA, 76% of these defects were restored to normal (Fig. **1J**, **1J'** and **1K**). These results demonstrate that alterations in the gene expression of *fhl1A* are associated with morphologic deformities in the somites and skeletal muscles.

3.2. Effects of *fhl1A* on the Expression of Skeletal Myogenesis Markers

Previous studies have shown that contractile muscles are derived from several populations of skeletal muscle precursors in somites [39]. The specific tempo-spatial expression pattern of *fhl1A* raises the question of whether it plays any role in skeletal myogenesis. Expression of m*yoD* takes place in the somites and adaxial cells and plays a central role in the induction of myogenic differentiation [36]. We used whole-mount *in situ* hybridization analysis to characterise the effects of *fhl1A* knockdown and overexpression on *myoD* expression during skeletal myogenesis.

The results demonstrated that, compared with control embryos (Fig. **2A**), *fhl1A* knockdown (Fig. **2B**) and overexpression (Fig. **2C**) had no effect on *MyoD* expression at the 3-somite stage, excepting a faint curvature of the axis (Fig. **2E**). At the 12-somite stage, compared with control embryos (Fig. **2F**), *fhl1A* knockdown led to a reduction of *myoD* expression in 82% of embryos (Fig. **2G**, **2J**), but *fhl1A* overexpression resulted in a broader and stronger *myoD* expression in 78% of embryos (Fig. **2H**, **2J**). When *fhl1A* MO and *fhl1A*-capped mRNA were co-injected, 89% of the embryos showed near normal expression of *myoD* (Fig. **2I**, **2J**). The question arose whether the protein level in skeletal muscle development was affected by *fhl1A*. At the 12-somite stage, 89% of *fhl1A* knockdown embryos showed a narrow expression of muscle fibres (Fig. **2L**, **2O**) relative to controls (Fig. **2K**), and 89% of *fhl1A* overexpression embryos exhibited broader expression domains of muscle fibres (Fig. **2M**, **2O**, arrowhead

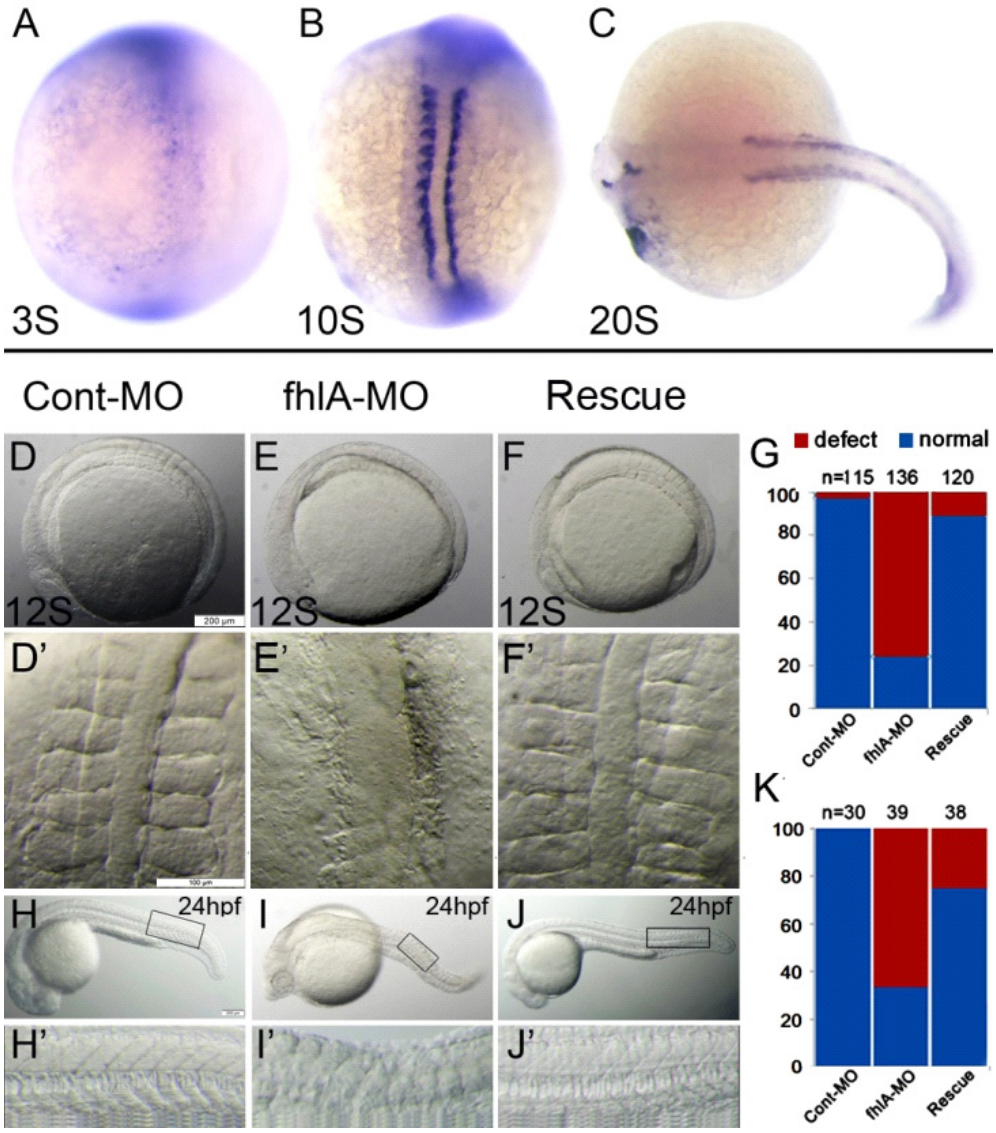

Fig. (1). Expression of *fhl1A* and its role in skeletal muscle development. (A) At the 3-somite stage, no *fhl1A* signal was detected in somites. **(B, C)** Specific expression of *fhl1A* in somites at the 10- and 20-somite stages. **(D, H)** Control embryos. **(E, I)** Knockdown of *fhl1A* resulted in defective somites. **(F, J)** *fhl1A* mRNA successfully recovered the *fhl1A* morphant. **(D', E', F', H', I' and J')** Partial amplifications of D, E, F, H, I and J, respectively. **(G, K)** Statistical analysis of D–F and H–J, respectively. D–F and H–J scale bars: 200 μm; D'–F' scale bar: 100 μm. The number of embryos analysed is shown in the columns.

indicated), which is consistent with the phenotypes detected by whole-mount *in situ* hybridization analysis. A similar phenotype was observed at the 25-somite stage, during which anti-MF20 antibody staining indicated that the knockdown of *fhl1A* was associated with defective expression of muscle fibres (Fig. **S1**). At 24 hpf, *fhl1A* depletion abolished the expression of *myoD* (71%, Fig. **2Q**, **2X**). This was further confirmed by the analysis at 28 hpf (81%, Fig. **2U**, **2Y**). In embryos co-injected with *fhl1A* mRNA, *myoD* expression was robust (65%, Fig. **2R**, **2X**). This was further confirmed by the analysis at 28 hpf when delayed disappearance of *myoD* relative to control embryos was observed in the tails of the injected embryos (79%, Fig. **2V**, **2Y**). Recovery of *myoD* expression was observed in 93% of embryos (Fig. **2S**, **2X**). These data support the conclusion that *fhl1A* affects skeletal muscle development; more specifically, *fhl1A* may delay the degradation of skeletal muscle differentiation marker, *myoD*.

3.3. Effects of *fhl1A* on Satellite Cell Formation

Previous reports have shown that satellite cells are progenitors of skeletal muscles and that the satellite cell marker *pax7* is expressed in the anterior part of somites during the 14-somite stage [40]. We examined whether *fhl1A* expression regulates the formation of satellite cells by first evaluating its effects on *pax7* mRNA levels using RT-PCR analysis. The results demonstrated that, in *fhl1A* knockdown embryos, the mRNA expression of *pax7a* and *pax7b* was significantly decreased at 24 hpf (Fig. **3A**, **3B**, $p < 0.01$). These results were further confirmed by immunostaining with anti-Pax7 antibody, which demonstrated that the number of Pax7-positive satellite

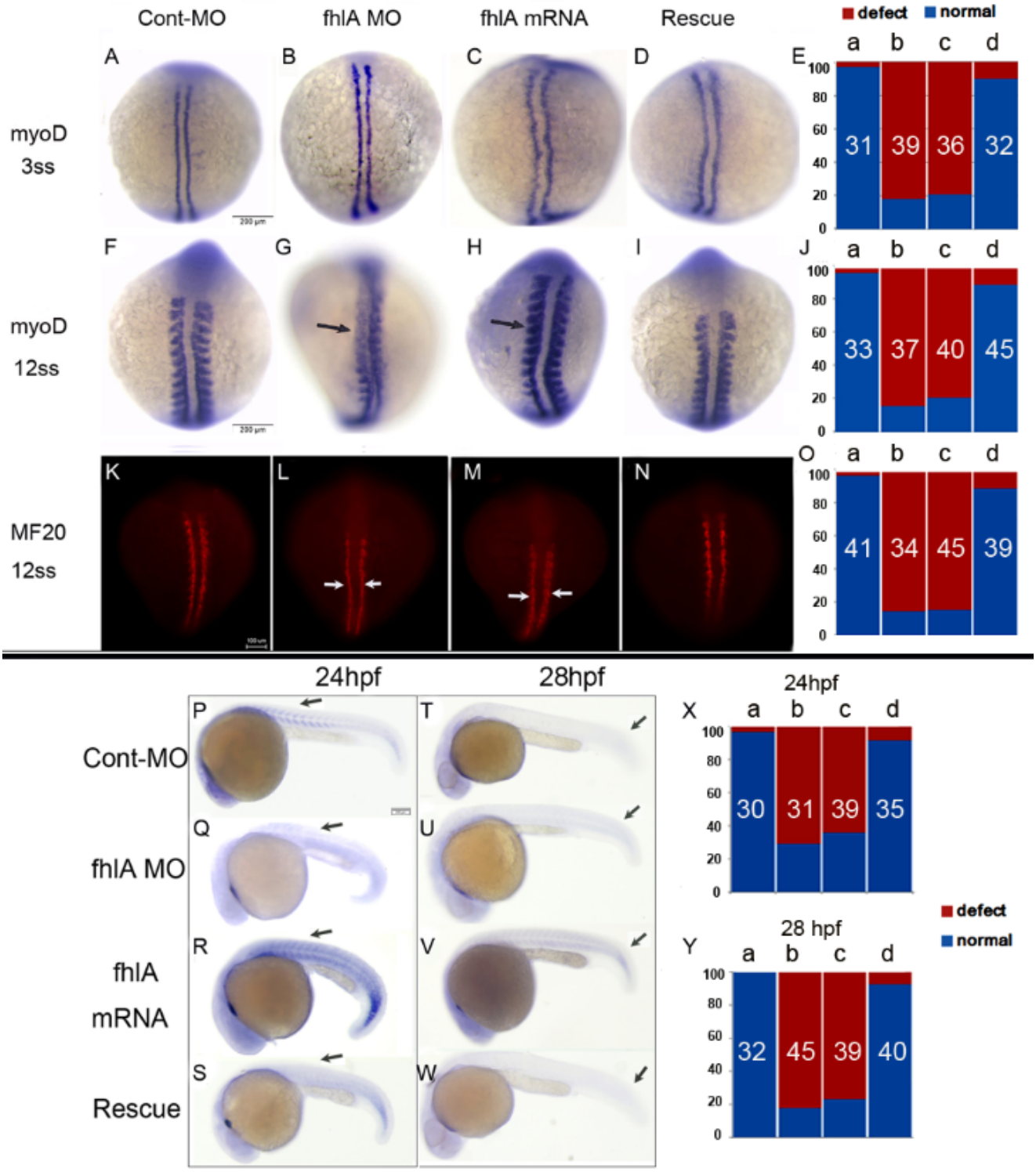

Fig. (2). Role of *fhl1A* in skeletal muscle development. (A–I) Embryos stained with *myoD* probes. (A–D) *fhl1A* had no effect on *myoD* at the 3-somite stage. (F–I) At the 12-somite stage, the expression of *myoD* in somites was upregulated. (H) Overexpression and downregulation of *fhl1A*. (G) Knockdown of *fhl1A* (arrowhead). **(E, J)** Statistical data of (A–D) and (F–I), respectively. Scale bars: 200 μm. **(K–N)** At the 12-somite stage, anti-MF20 antibody was used for immunostaining, suggesting skeletal muscle was regulated by *fhl1A*: (K) Control-MO embryos, (L) *fhl1A* knockdown embryos, (M) embryos injected with *fhl1A* mRNA, (N) embryos co-injected with *fhl1A* MO and *fhl1A* mRNA. **(O)** Statistical data of (K–N). Scale bar: 100 μm. **(P–W)** The expression of *myoD* disappeared from somites in (P) Control-MO and (Q) *fhl1A* MO and from (S) recovered embryos, but *myoD* was still expressed in (R) embryos overexpressing *fhl1A* (arrowhead). **(X, Y)** Statistical data of (P–S) and (Q–T), respectively. The symbols a–d represent control, *fhl1A* MO, *fhl1A* mRNA and Rescue, respectively. The number of embryos analysed is shown in the columns.

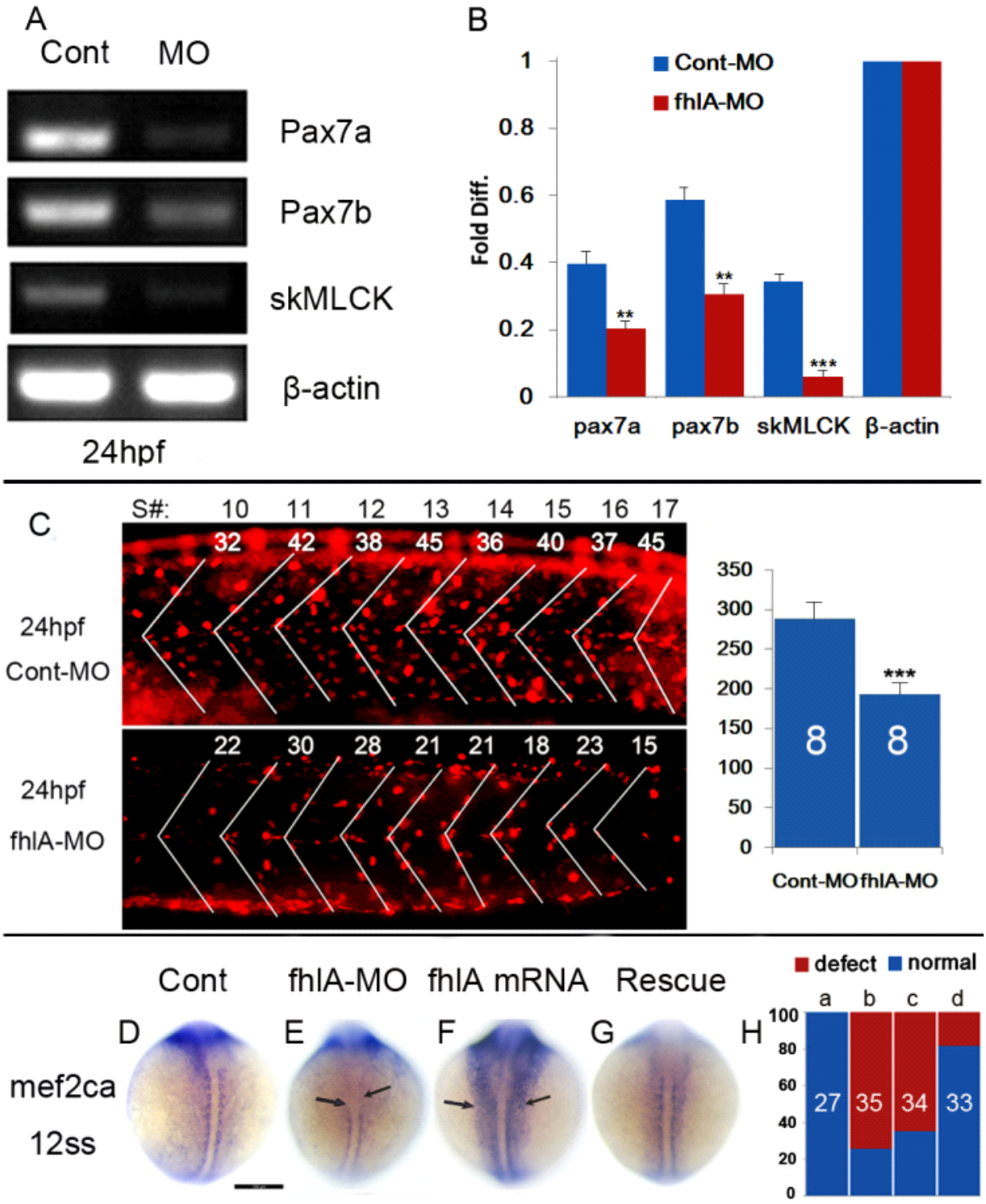

Fig. (3). Role of *fhl1A* in satellite cells. (A) At 24 hpf, RT-PCR results demonstrated that satellite cell markers were severely downregulated when *fhl1A* was knocked down. **(B)** Statistical data from A; experiments were repeated at least 3 times. **(C)** Expression of *fhl1A* was required for formation of satellite cells. At the 10- to 17-somite stage, the number of Pax7^{+} myogenic precursors in *fhl1A* knockdown embryos was significantly decreased. Anterior to the left, dorsal up. **(D–G)** Expression of *fhl1A* was found to regulate somite development. Somitic expression of *mef2ca* was (F) upregulated by *fhl1A* overexpression and (E) downregulated as *fhl1A* was knocked down (arrowhead). (D) Control embryos. (G) Embryos co-injected with *fhl1A* MO and *fhl1A* mRNA. **(H)** Statistical data of (D–G). The symbols a–d represent control, *fhl1A* MO, *fhl1A* mRNA and recovered, respectively. The number of embryos analysed is shown in the column, (** $p < 0.01$, *** $p < 0.001$). Error bars indicate ± s.e.m.

cells at 24 hpf was significantly lower ($p < 0.001$) in *fhl1A* knockdown embryos (with an average of 183 cells counted from somitic 10 to 17, n = 8) than in controls (with an average of 279 cells, n = 8) as shown in Fig. **3C**, suggesting that *fhl1A* affected the number of satellite cells.

Previous studies have shown that *mef2c* is highly expressed in quiescent satellite cells and during satellite cell myogenesis [41]. They have established that skeletal muscle commitment takes place through the amplification of and synergism with *myoD* [42, 43]. We examined whether skeletal muscle development affected by *fhl1A* could bypass *mef2ca*, a zebrafish homologue of human *mef2c*, at the 12-somite stage. In *fhl1A* knockdown embryos, *mef2ca* expression was reduced in anterior somites (Fig. **3E**, arrowhead indicated), with 77% of the embryos showing defective *mef2ca* expression (Fig. **3H**). However, *fhl1A* overexpression caused upregulation of *mef2ca* expression in somites, with high ectopic expression in a bilateral pattern along the somitic paraxial mesoderm. This took place in 65% of embryos with defective phenotypes (Fig. **3F**, **3H**). These *mef2ca* expression phenotypes were recovered in 82% of the *fhl1A*-MO embryos co-injected with *fhl1A* mRNA (Fig. **3G**, **3H**). Recently, skMLCK was found to regulate *myoD* and *myf5* expression in ES and satellite cells, and the specificity of *skMLCK* regulating activities may be mediated by the ability of *mef2c* to recruit p300 to skeletal muscle promoters during muscle commitment [27]. We explored whether expression of *fhl1A* affects the expression of *skMLCK* in skeletal myogenesis. As shown in Fig. **3A** and **3B**, the mRNA expression level of *skMLCK* was significantly decreased in *fhl1A* knockdown embryos ($p < 0.001$). These results were further confirmed by whole-mount in situ hybridization, which showed that *skMLCK* expression was significantly reduced in 90% of *fhl1A* knockdown embryos relative to controls (Fig. **S2**). These data support the observation that *fhl1A* plays a role in regulating satellite cells for skeletal muscle development.

3.4. Effects of RA Signalling on *fhl1A* Expression and Skeletal Muscle Development

As the expression of *fhl1A* is regulated by RA signalling, we proposed that the role of *fhl1A* in skeletal muscle development could be modulated by RA signalling [37]. We analysed *fhl1A* expression during myogenesis at the 10-somite stage. In the presence of exogenous RA, higher levels of *fhl1A* expression were induced in somites than in controls (90%, n = 76, Fig. **4J**, **4N**). In contrast, *fhl1A* expression was lower in somites in the presence of exogenous DEAB (89%, n = 65 Fig. **4K**, **4N**) than in controls (n = 70, Fig. **4I**). Next, we monitored the protein levels of Fhl1A after treatment with RA and DEAB, and under these conditions, the endogenous Fhl1A was upregulated and downregulated, respectively (Fig. **S3**). These data suggest that endogenous *fhl1A* expression in skeletal muscles is influenced by RA signalling.

We next determined whether the *myoD* expression in somites could be modulated by RA signalling. As expected, the *myoD* expression in the somites of RA-treated embryos at the 10- and 16-somite stages (98%, Fig. **4B**, **4F**, **4L** and **4M**) was higher than in controls (100%, Fig. **4A**, **4E**), but the expression of *myoD* in adaxial cells appeared to be unchanged. In DEAB-treated embryos, *myoD* expression was reduced in somites (93% Fig. **4C**, **4G**), similar to results observed in *fhl1A* morphant embryos (Fig. **4D**, **4H**). Phenotypes in the somites regulated by RA signalling resembled those of *fhl1A* knockdown and overexpression embryos (Fig. **2G**, **2H**), suggesting that *fhl1A* expression has effects comparable to those of RA signalling in myogenesis. These data collectively point to a role of *fhl1A* in specifying embryonic skeletal muscle downstream of RA signalling in zebrafish.

4. DISCUSSION

In the present study, we demonstrated that *fhl1A* is specifically expressed in skeletal muscles during the 8-, 12- and 20-somite stages. We provide evidence that the expression of *fhl1A* plays a pivotal role in the development of skeletal muscle.

To examine the effects of *fhl1A* loss of function on skeletal muscle development, we used *fhl1A* MO to knock down Fhl1A protein expression. The effectiveness of *fhl1A* MO used in this analysis was demonstrated in our previous paper, in which we examined the *fhl1A-GFP* expression in zebrafish embryos by detecting Fhl1A-GFP fluorescence and analysed the Fhl1A protein expression by western blot. These results suggest that *fhl1A* MO specifically blocks the expression of the *fhl1A* gene *in vivo* [37]. In the present study, we demonstrated that the tempo-spatial specific expression of *fhl1A* had associated effects on skeletal muscle development. During the 12- through 18- somite stages, *fhl1A* expression was only detectable in the skeletal muscle cells, suggesting that *fhl1A* impacts the development of skeletal muscle. Knockdown of *fhl1A* caused defective skeletal muscle development. These defects were found to be largely recovered in *fhl1A* morphants co-injected with *fhl1A* mRNA. These results suggest that *fhl1A* is required for skeletal muscle development.

Fhl1A directly activates the expression of myogenic regulatory factors *myoD*, *myf5*, *mrf4* and *myogenin*, thus activating a skeletal muscle differentiation gene expression programme [44, 45]. We demonstrated that the expression of *myoD* is significantly reduced in *fhl1A* knockdown embryos (Fig. **2G**), and that *fhl1A* overexpression led to an increased expression of *myoD* (Fig. **2H**). We further analysed *myoD* expression at 24 hpf and found it to be minimal. No *myoD* expression was detected in *fhl1A* knockdown embryos (Fig. **2Q**), while expression was delayed in embryos overexpressing *fhl1A*. Abnormal expression of *myoD* could be corrected at 24 phf (Fig. **2S**), with similar results observed at 28 hpf (Fig. **2W**).

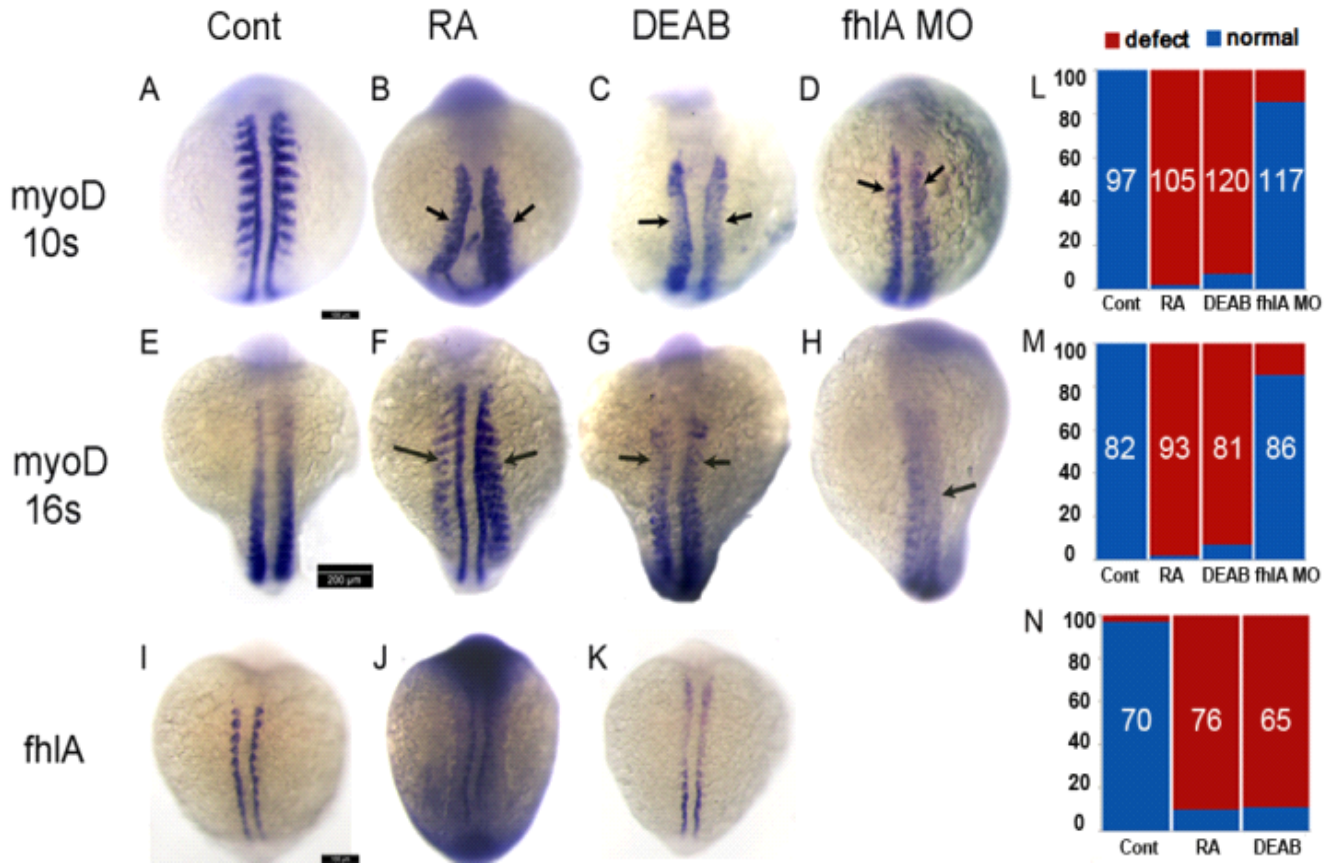

Fig. (4). Effects of RA signalling on endogenous *fhl1A* expression. (A–H) Phenotypes obtained by whole-mount *in situ* hybridization with *myoD* antisense RNA probe. (B, F) The expression of *myoD* was upregulated in the presence of exogenous RA and (C, G) downregulated by exogenous DEAB. (D, H) *fhl1A* MO resulted in downregulated expression of *myoD*. Black arrows indicate the expression of *myoD* at somites. **(I–K)** Endogenous expression of *fhl1A* was found to be regulated by RA. **(L, M)** Statistical data from A–D and E–H, respectively. The number of embryos analysed is shown in the columns.

Satellite cells are progenitor cells that generate the myoblasts required for muscle growth, repair and regeneration [21]. *Mef2c* is expressed at all stages of satellite cell myogenesis and is required for the development of skeletal muscle [26]. Expression of *skMLCK* is important for the activation of satellite cells and their subsequent differentiation [27]. Our results demonstrated that, at 24 hpf, knockdown of *fhl1A* produced significant downregulation of the expression of satellite cell markers *pax7a* and *pax7b*, and the regulation genes *mef2ca* and *skMLCK*, suggesting that *fhl1A* plays a pivotal role in the formation of satellite cells. Our results further demonstrated that *fhl1A* expression in somites is controlled by RA signalling. Previous studies have shown that RA signalling activates the expression of fast muscle myosin by inducing premature *myoD* expression in the presomitic mesoderm, but that DEAB represses it by reducing expression of *myoD* in somites [46]. Our results demonstrated that both *fhl1A* expression and RA signalling produced a similar phenotype, suggesting that the role of *fhl1A* in skeletal muscle development is likely regulated by RA signalling. Collectively, these results demonstrate that the *fhl1A* gene functions as an activator of skeletal muscle development in living organisms.

Mutations in the *FHL1* gene have been identified in a number of human myopathies. In the present study, we demonstrated that skeletal muscle defects occur after alterations in *fhl1A* expression, indicating that the zebrafish *fhl1A* mutation model may be suitable for studying the effects of genetic variations identified in patients with myopathies.

CONCLUSION

In summary, our results demonstrate that *fhl1A* plays pivotal roles in regulating the number of satellite cells and skeletal muscle development. However, a complete understanding of the specific molecular mechanism by which *fhl1A* promotes skeletal myogenesis requires further investigation.

ABBREVIATIONS

DEAB = Diethylaminobenzaldehyde

MO = Morpholino

RA = Retinoic acid

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Full details of the study were approved by Ethics Committee of Hunan Normal University.

HUMAN AND ANIMAL RIGHTS

No humans were used for studies that are base of this research. All embryos were handled according to relevant national and international guidelines “Act on Welfare and Management of Animals”.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

This study was supported in part by grants from the National Natural Science Foundation of China (Nos.: 81470449, 81470377, 81670290, 31572349, 81370451, 81400304, 81270156, 81370230, 81570279; 81700338, 81270291, 81670288, 81370341), the Cooperative Innovation Center of Engineering and New Products for Developmental Biology of Hunan Province (No. 2013-448-6), China National Ministry of Science and Technology key national research and development plan: SQ2017ZY050117, Guangdong Provincial Natural Science Foundation project: 1714060000079, and Guangzhou Science and Technology Plans major projects of Production-study-research cooperative innovation: 201508020107.

SUPPLEMENTARY MATERIAL

Supplementary material is available on the publisher's website along with the published article.

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