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RESEARCH ARTICLE

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Interferon regulatory factor-7 is required for hair cell development during zebrafish embryogenesis

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Abstract

Interferon regulatory factor-7 (IRF7) is an essential regulator of both innate and adaptive immunity. It is also expressed in the otic vesicle of zebrafish embryos. However, any role for *irf7* in hair cell development was uncharacterized. Does it work as a potential deaf gene to regulate hair cell development? We used whole-mount in situ hybridization (WISH) assay and morpholino-mediated gene knockdown method to investigate the role of *irf7* in the development of otic vesicle hair cells during zebrafish embryogenesis. We performed RNA sequencing to gain a detailed insight into the molecules/genes which are altered upon downregulation of *irf7*. Compared to the wild-type siblings, knockdown of *irf7* resulted in severe developmental retardation in zebrafish embryos as well as loss of neuromasts and damage to hair cells at an early stage (within 3 days post fertilization). Coinjection of zebrafish *irf7* mRNA could partially rescued the defects of the morphants. *atp1b2b* mRNA injection can also partially rescue the phenotype induced by *irf7* gene deficiency. Loss of hair cells in *irf7*-morphants does not result from cell apoptosis. Gene expression profiles show that, compared to wild-type, knockdown of *irf7* can lead to 2053 and 2678 genes being upregulated and downregulated, respectively. Among them, 18 genes were annotated to hair cell (HC) development or posterior lateral line (PLL) development. All results suggest that *irf7* plays an essential role in hair cell development in zebrafish, indicating that *irf7* may be a member of deafness gene family.

KEYWORDS
embryogenesis, hair cells, interferon regulatory factor-7, morpholinos, zebrafish

1 | INTRODUCTION

Currently, around 466 million people worldwide have disabling hearing loss, and this figure is estimated to rise to over 900 million by 2050 (Kanzaki, 2018). Causes of hearing loss are multifactorial, and the damage of mechanosensory hair cells (HCs) in the inner ear is one of the most common mechanisms (Cunningham & Tucci, 2017). Degeneration and death of HCs may lead to permanent hearing loss in humans since they cannot regenerate in adult mammals (Chen et al., 2019; Liu et al., 2014; Sotomayor et al., 2012). The morphogenesis of inner ear shares a high degree of similarity among vertebrates (Bever & Fekete, 2002). Zebrafish (*Danio rerio*) have plenty of hair cells in their inner ear and lateral

line, and they are considered as an excellent animal model to investigate the hair cell development. There were more and more genes or signaling pathways being proved to play vital roles in zebrafish hair cell development, such as *atoh1a* (Sarrazin et al., 2006), *cib2* (Riazuddin et al., 2012), *myo6b* (Kappler et al., 2004; Seiler et al., 2004), *tmie* (Gleason et al., 2009; Pacentine & Nicolson, 2019; Shen et al., 2008), *rbm24a* (Cheng et al., 2020; Zhang et al., 2020), *kremen1* (Mulvaney et al., 2016) and JNK pathway (Cai et al., 2016). In addition, zebrafish have the ability to regenerate the injured tissues. Therefore, they have been widely used in studying the regeneration of heart, retina, spinal cord, caudal fin and appendages (Hui et al., 2010; Lu et al., 2016; Sherpa et al., 2008; Shi et al., 2021; Yin & Poss, 2008). They also show a capacity for hair cell regeneration (Lush & Piotrowski, 2014; Monroe et al., 2015). Therefore, the zebrafish model is appropriate for addressing questions related to hair cell development and regeneration.

Interferon regulatory factors (IRFs) are a family of transcription factors with pivotal roles in the immune response (Jefferies, 2019). As one of the members, IRF7 was first identified in the context of latent Epstein–Barr virus (EBV) infection and is an essential regulator of both innate and adaptive immunity (Ning et al., 2011). Based on the prediction from ZFIN (https://zfin.org/ZDB-GENE-040426-1518), *irf7* was found expressed in the otic vesicle of zebrafish embryos. However, the role of *irf7* in hearing remains to be elucidated. The current study first examined *irf7* mRNA production in zebrafish embryos using a whole-mount in situ hybridization (WISH) assay and revealed a novel role of *irf7* in HC development, which may contribute to our understanding of hearing loss and provide potential strategies for deafness therapy.

2 | MATERIALS AND METHODS

2.1 | Ethics statement

All animal experimentation was carried out in accordance with NIH Guidelines for the care and use of laboratory animals (http://oacu.od.nih.gov/regs/index.htm) and ethically approved by the Administration Committee of Experimental Animals, Jiangsu Province, China (Approval ID: SYXK(SU) 2007−0021). Best efforts were made to minimize the number of animals used and prevent their suffering.

2.2 | Zebrafish husbandry and strains

Wild-type adult zebrafish (AB line) were supplied by the Zebrafish Center at Nantong University, Jiangsu Key Laboratory of Neuroregeneration. A transgenic line, *Tg(Brn3c:GFP)* in which HCs were specifically labeled by green fluorescent protein (GFP) was used in this study (Xiao et al., 2005). Zebrafish embryos and adult fish were raised and maintained under conditions previously described (Krueger et al., 2011).

2.3 | Whole-mount in situ hybridization

WISH with an antisense RNA probe was performed according to a standard protocol. The template for the probe to detect the expression of *irf7* was cloned from the corresponding cDNA fragment. Part of the coding sequence of the *irf7* gene was amplified and subcloned into a pGEM-T Easy vector. Amplification of *irf7* was achieved using a forward primer: 5′-CTTGGTGACGCGGATGTTAA-3′ and reverse primer: 5′-GCATGCATGGATCGTCTGAA-3′. The RNA probe for detecting *eya1* was kindly provided by Dr. Y. He from Fudan University (Shanghai, China). The digoxigenin-labeled RNA probe was transcribed in vitro using DIG RNA Labeling Kit (Roche, USA) according to the manufacturer's instructions. For hybridization, the prefixed embryos were first incubated with the RNA probe at 4˚C overnight. Then, the alkaline phosphatase-conjugated antibody against digoxigenin (Roche, USA) was added to interact with the digoxigenin-labeled RNA probe. Finally, NBT/BCIP solution (substrate for alkaline phosphatase, Roche) was added to elicit a chromogenic reaction, and the development of the expected color allowed the expression of the target gene to be observed. After hybridization, an Olympus stereomicroscope MVX10 equipped with an Olympus DP71 camera was used to produce images of the embryos.

2.4 | Knockdown of *irf7* gene in zebrafish embryos with antisense morpholino

The sequence of the *irf7* morpholino antisense oligomer was 5′-ATGCATGGAGGAAGTTACCTTGAA-3′ and was supplied by Gene Tools, LLC. Each embryo was injected with 2–3 nl of 0.3 mM morpholino oligo at the one-cell stage. Morpholino blocked the splicing of *irf7* mRNA.

2.5 | Generation of *irf7*-mutant zebrafish using CRISPR/Cas9 technology

As previous mentioned (Qian et al., 2020), Cas9 mRNA and *irf7*-specific single guide RNA (sgRNA) were synthesized in vitro and then coinjected into the one-cell-stage zebrafish embryos. Then, the injected embryos were genotyped and the mutant zebrafish were used to perform the phenotypic analysis. Here, the sequence of target site of *irf7*-sgRNA is 5′-GTGGAAAGTGGGCAGTACGA-3′, which is located in the second exon of *irf7* gene.

2.6 | CRISPRi-mediated gene knockdown of *atp1b2b*

The CRISPRi system (Long et al., 2015; Qi et al., 2013) was used to repress the *atp1b2b* gene expression. Briefly, dCas9 (catalytically dead Cas9 lacking endonuclease activity) mRNA together with *atp1b2b*-specific sgRNA were coinjected into the one-cell-stage zebrafish embryos to block the transcriptional elongation of target gene. Here, the target of *atp1b2b*-sgRNA-binding was 5′-GGGAGGACCGCGAGCAGTTG-3′.

2.7 | Real-time quantitative PCR

Total RNA was extracted from zebrafish embryos and then reversely transcribed into cDNA. The real-time quantitative PCR analysis was performed according to the procedure. The primers used for *irf7* gene were 5′-TCGCATCATTCGTCCTCAAA-3′ and 5′-AGGTCTTCAGTGACTGGGAA-3′; for *eya4* gene were 5′-CCCACTGCCCCAAGTATGAA-3′ and 5′-CCTGTGTAGGCTGACATGGG-3′.

2.8 | Rescue experiment

To reverse the effects of *irf7* knockdown, 2–3 nl of 100 ng/μl *irf7* mRNA was coinjected with *irf7* antisense morpholino into zebrafish embryos. To make the RNA, the coding sequence of *irf7* was first amplified by PCR (forward primer: 5′-GGAATTCGAAAGTACGTGGTAGTAGCG-3′ and reverse primer: 5′-GCTCTAGAGAGATTAAATGCCAGAAACA-3′) and cloned into pCS2+ vector. The recombinant DNA plasmid was then linearized by cutting with an appropriate restriction enzyme and transcribed into RNA in vitro using the mMessage mMachine T7 kit (Ambion, UK). Likewise, in the *atp1b2b* rescue experiment, the PCR primers used to amplify the coding sequence of *atp1b2b* gene are 5′-CCCGAGGAGTTGTCTCTGTG-3′ and 5′-TTAGATGGGAGGACCGGTGT-3′, respectively.

2.9 | Detection of apoptosis

Apoptosis in *irf7*-morpholino and control-morpholino-injected zebrafish embryos was analyzed with 48 hpf, and TUNEL staining was performed according to the manufacturer's instructions (Cell death detection kit, Roche).

2.10 | Confocal imaging

For confocal imaging of HCs and neuromasts, *irf7*-morpholino and control-morpholino-injected *Tg(Brn3c:GFP)* embryos were anaesthetized with egg water/0.16 mg/ml, tricaine/1%, 1-phenyl-2-thiourea (Sigma, USA) and embedded in 0.6% low melting agarose. Live imaging was performed with Nikon A1R confocal microscope.

2.11 | mRNA sequencing by Illumina HiSeq

Whole mRNA sequencing of *irf7*-morpholino-injected and control zebrafish embryos (72 hpf) was performed using GENEWIZ (GENEWIZ, Inc., USA). Total RNA of each sample was extracted with TRIzol Reagent (Invitrogen, USA) and quantified and qualified by NanoDrop (ThermoFisher Scientific, USA) and 1% agarose gel. Note that 1 μg RNA was used for library preparation. Next, generation sequencing library preparations were constructed according to the manufacturer's protocol. The poly(A) mRNA isolation was performed using Poly(A) mRNA Magnetic Isolation Module or rRNA removal Kit. The mRNA fragmentation and priming were performed using First Strand Synthesis Reaction Buffer and Random Primers. First, strand cDNA was synthesized using ProtoScript II Reverse Transcriptase, and second-strand cDNA was synthesized using Second Strand Synthesis Enzyme Mix. The bead-purified double-stranded cDNA was then treated with End Prep Enzyme Mix to repair both ends and add dAtail, followed by a T-A ligation to add adaptors to both ends. Size selection of adaptor-ligated DNA was then performed using beads, and fragments of ~420 bp (with the approximate insert size of 300 bp) were recovered. Each sample was then amplified by PCR for 13 cycles using P5 and P7 primers, with both primers carrying sequences which can anneal to the flow cells to perform bridge PCR and P7 primers carrying a six-base index allowing for multiplexing. The PCR products were cleaned up using beads, validated using Qsep100 (BiOptic Inc., Taiwan, China) and quantified by Qubit3.0 Fluorometer (Invitrogen). Libraries with different indices were multiplexed and loaded on an Illumina HiSeq instrument according to the manufacturer's instructions (Illumina, USA). Sequencing was carried out using a 2 × 150 bp paired-end (PE) configuration; image analysis and base calling were conducted by the HiSeq Control Software (HCS) + OLB + GAPipeline-1.6 (Illumina) on the HiSeq instrument. The sequences were processed and analyzed by GENEWIZ.

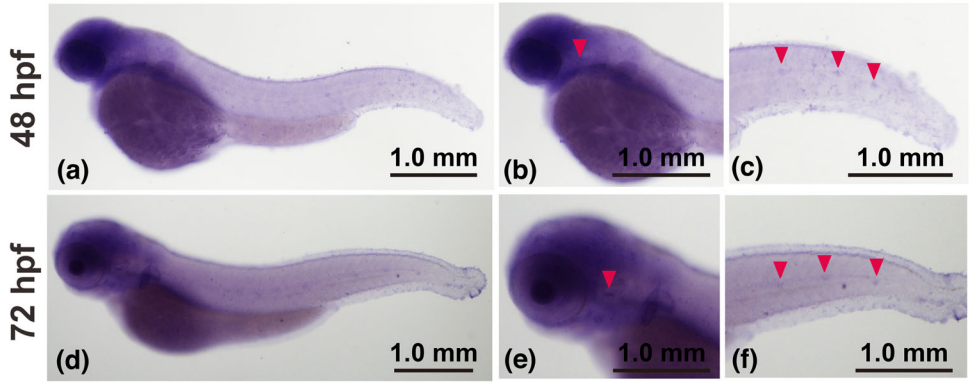

FIGURE 1 Expression of *irf7* in zebrafish larvae. *irf7* expression was detected with whole-mount in situ hybridization at 48 hpf (a–c) and 72 hpf (d–f), respectively. The *irf7* mRNA was expressed in the otic vesicle and lateral line (indicated by red arrowhead)

2.12 | Data analysis

The number of lateral line neuromasts and the number of HCs in the inner ear were counted manually. All data were analyzed using GraphPad Prism 8.0. A two-tailed, unpaired Student's *t*-test was used to determine statistical significance when comparing independent groups. $P < 0.05$ was considered statistically significant. All data are presented as mean ± SD, and each experiment comprised at least three replicates.

3 | RESULTS

3.1 | Zebrafish *irf7* gene is expressed in otic vesicles and lateral line neuromasts

The WISH assay was performed to detect the expression of *irf7* in developing zebrafish embryos. The *irf7* mRNA transcript was observed in the otic vesicle and lateral line at 48 and 72 hpf (Figure 1). Moreover, its expression was detected in the lateral line neuromasts at both time points (Figure 1c,f). These results indicated that the *irf7* gene was expressed in neuromasts, suggesting a potential role for *irf7* in HC development.

3.2 | Knockdown of *irf7* leads to reduced numbers of neuromasts and HCs

To explore the effects of *irf7* on HC development, morpholino-mediated gene knockdown was utilized to downregulate *irf7* expression in a transgenic line *Tg(Brn3c:GFP)* in which HCs were fluorescently labeled by GFP. The effect of the morpholino-induced splicing block was examined with reverse transcriptase (RT)-PCR (Figure S1). To do this, mRNAs of the control and morpholino-injected embryos were extracted and reverse transcribed into cDNA in vitro, respectively. Furthermore, PCR with primers complementary to the first exon and the third exon of *irf7* was used to amplify the sequence between the two exons. Since morpholino recognizes and binds to the end of the second exon, it results in an incorrect identification of the second exon as a part of the intron. Thus, the second exon is excised during pre-mRNA splicing resulting in PCR products that are shorter than those of the control (Figure S1). Zebrafish embryos injected with the *irf7*-specific morpholino displayed slight developmental retardation and curvature of the spine during the early stage compared with embryos injected with control morpholino (Figure 2a). However, the morphants recovered to display the normal phenotype by 5 days post fertilization (dpf) (data not shown). Fluorescence imaging of the whole zebrafish larvae further showed that the numbers of HC clusters in the lateral line of *irf7* morphants were far fewer than those of the control at different developmental stages (Figure 2b,c). To further confirm that the decreased number of neuromasts was the result of *irf7* gene knockdown, we performed WISH with an RNA probe specific for the *eya1* gene, which has been identified as a marker gene of neuromast cells in zebrafish lateral line (Kozlowski et al., 2005; Sahly et al., 1999). Results from the WISH assay indicated that the number of lateral line neuromasts was significantly reduced in *irf7*-knockdown zebrafish (Figure S2).

3.3 | Downregulation of *irf7* inhibits the development of HCs

As we observed before, knockdown of the *irf7* gene led to decreases in neuromasts and HC clusters. However, whether the HCs in the remaining neuromasts were also influenced by downregulation of *irf7* was still unclear. Here, we performed confocal imaging on *irf7*-specific morpholino-injected *Tg(Brn3c:GFP)* zebrafish embryos to visualize the HCs in the remaining neuromasts. Compared with the control

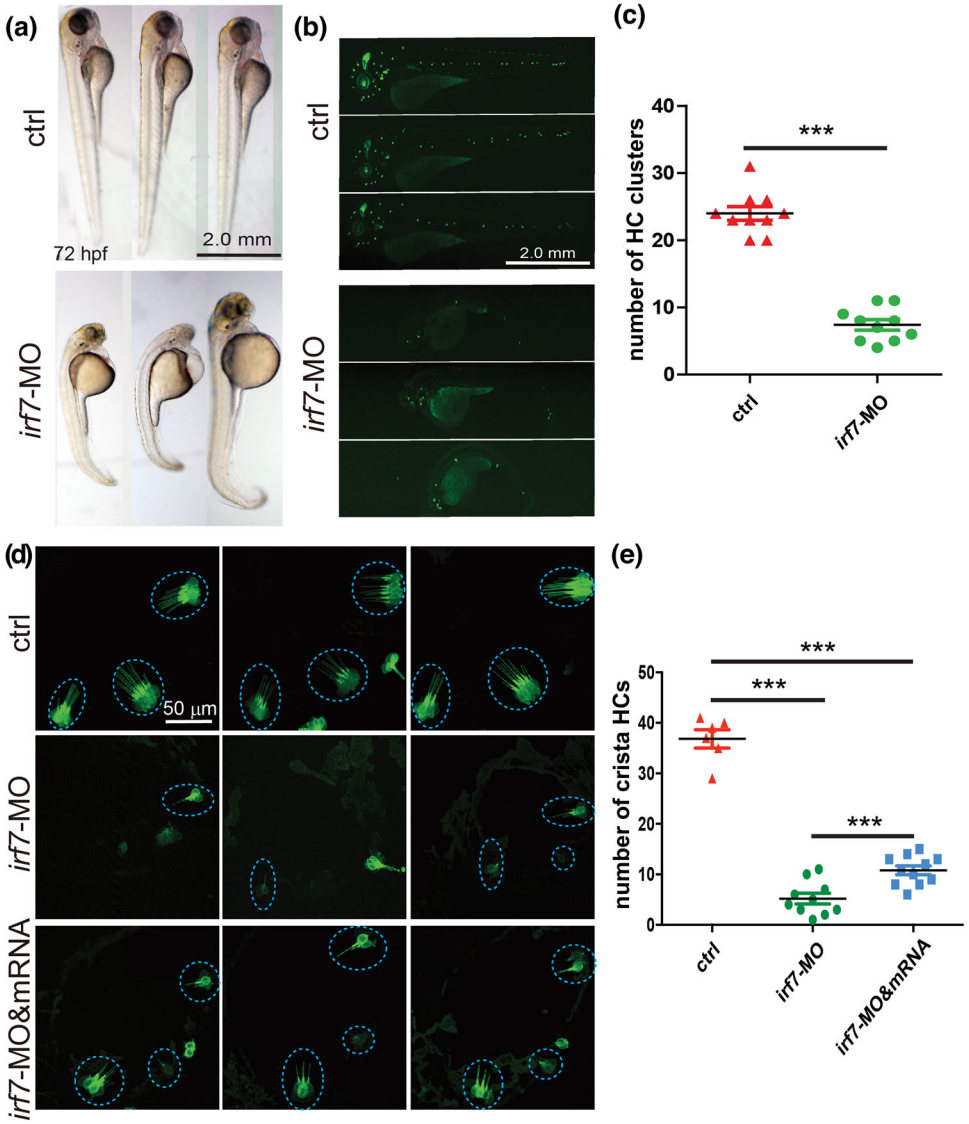

FIGURE 2 *irf7* knockdown zebrafish embryos exhibit severe defects in hair cell (HC) development. (a) Phenotypes of control and *irf7*-specific morpholino-injected *Tg(Brn3c:GFP)* zebrafish larvae at 72 hpf. (b) Fluorescent imaging of green fluorescent protein (GFP)-labeled HCs indicates the HC clusters on the lateral line of zebrafish larvae at 72 hpf. (c) Quantification of the number of HC clusters in control and *irf7*-specific morpholino-injected *Tg(Brn3c:GFP)* zebrafish embryos at 72 hpf. (d) Comparison of the phenotypes of crista HCs (blue dotted circles) in control, *irf7*-specific morpholino-injected and *irf7*-specific morpholino & mRNA-coinjected zebrafish embryos. (e) Quantification of the number of crista HCs in control, *irf7*-specific morpholino-injected and *irf7*-specific morpholino & mRNA-coinjected zebrafish embryos. Error bars indicate standard deviation. ***, $P < 0.001$

morpholino-injected embryos, knockdown of *irf7* resulted in not only decreased HC cluster numbers but also morphological changes in HCs in the remaining crista neuromasts (Figure 2b–d). In an individual HC cluster of the remaining cristae neuromasts, the kinocilium length was much shorter in *irf7*-morphants (Figure 2d). Similarly, the *irf7*-mutant zebrafish, which were generated by CRISPR/Cas9 technology, also exist significant hair cell loss compared to the controls (Figure S3). Together, these results demonstrated that downregulation of *irf7* had a negative effect on HC development in zebrafish.

3.4 | *irf7* mRNA injection partially rescues the phenotype induced by *irf7*-knockdown

To test whether the phenotype of *irf7*-morphants was caused by the downregulation of the *irf7* gene, we performed a rescue experiment by coinjecting *irf7* mRNA with *irf7*-specific morpholino into one-cell-stage *Tg(Brn3c:GFP)* zebrafish embryos. The zebrafish embryos injected with *irf7* mRNA had more HCs in an individual neuromast than those without *irf7* mRNA injection (Figure 2e). In addition, the morphology

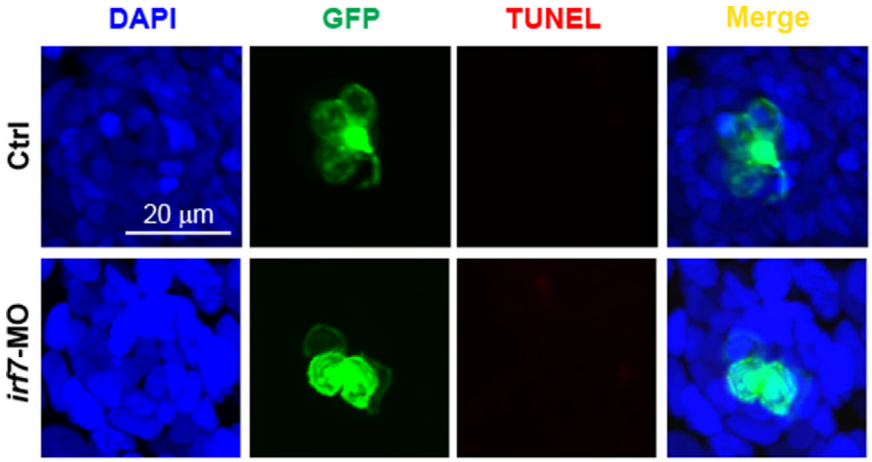

FIGURE 3 Apoptotic analysis of hair cells (HCs) in the neuromasts of control and *irf7*-knockdown embryos. DAPI, green fluorescent protein (GFP) and TUNEL (TMR red) were used to label the live cell nuclei, HCs and apoptotic HCs, respectively

of the HCs was also partially restored upon *irf7* mRNA injection and the kinocilia were longer (Figure 2d). Taken together, these results suggested an essential role of *irf7* in HC development in zebrafish.

3.5 | Loss of HCs in *irf7*-morphants does not result from cell apoptosis

A TUNEL staining experiment was performed to determine whether the decreased number of HCs in the neuromasts of *irf7*-morphants arose from HC apoptosis. TUNEL staining consists of a terminal deoxynucleotidyl transferase (TdT)-mediated enzymatic reaction which labels DNA strand breaks generated as a result of apoptosis. Apoptosis is thus distinguished from necrosis and from DNA strand breaks induced by cytostatic drugs or irradiation. The products of DNA degradation in apoptotic cells were labeled by the organic fluorophore, TMR red. The fluorescent dye, DAPI (4′,6-Diamidino-2-phenylindole dihydrochloride), was used to label double-stranded DNA for visualization of the nuclei. As shown in Figure 3, the GFP-labeled HCs in *Tg(Brn3c:GFP)* zebrafish larvae did not colocalize with TMR-labeled cells, indicating that *irf7* knockdown did not cause HC apoptosis.

3.6 | *atp1b2b* mRNA injection partially rescues the phenotype induced by *irf7* gene deficiency

To gain a detailed insight into the molecules/genes which are altered upon downregulation of *irf7*, RNA sequencing was performed. Gene expression profiles were compared between *irf7*-morphants and wild-type embryos at 72 hpf in three independent experiments. Compared to wild-type, knockdown of *irf7* can lead to 2053 and 2678 genes being upregulated and downregulated, respectively (Figure 4a). Among them, 18 genes were annotated to HC development or posterior lateral line (PLL) development, and the relative expression of them between wild-type and *irf7*-morphants was presented (Figure 4a–b). To confirm our observation, we injected *atp1b2b* mRNA together with *irf7* morpholino into wild-type zebrafish embryos. Overexpression of *atp1b2b* gene could partially rescue the phenotype induced by *irf7* gene deficiency (Figure 4c). Atp1b2b, one of the subunits of Na^+, K^+-ATPase, was reported to be regulated by Eya4 (Wang et al., 2008). Therefore, we detected the mRNA level of *eya4* and *irf7* in the *atp1b2b* gene knockdown zebrafish, which was generated through CRISPR interference (CRISPRi) method, and found that downregulation of *atp1b2b* did not alter the expression of *eya4* and *irf7* gene (Figure S4). These results demonstrate that transcription factor Irf7 is required for hair cell development, and it can regulate the Atp1b2b expression through direct or indirect ways in zebrafish (Figure 5).

4 | DISCUSSION

HCs in zebrafish neuromasts play a critical role in sensing signals within the surrounding environment and regulating a subset of behaviors, such as hearing movement, predator avoidance and prey detection (Dambly-Chaudiere et al., 2003). HC damage is a leading cause of hearing loss in humans since these cells are very rarely regenerated in mammals. In zebrafish, neuromasts are located at the lateral line and distributed in several regions at regular intervals from the head to the tip of the tail (Ghysen & Dambly-Chaudiere, 2004). The even distribution of neuromasts on the lateral line ensures highly abundant HCs in zebrafish and ensures the ability of the fish to perceive changes in its surroundings. Zebrafish HCs have similar morphology and functions to mammalian HCs in sensing and transmitting external signals. Thus, the zebrafish is an excellent model system for studies of HC development. It is also advantageous as an animal model because of its optical transparency, high rate of reproduction

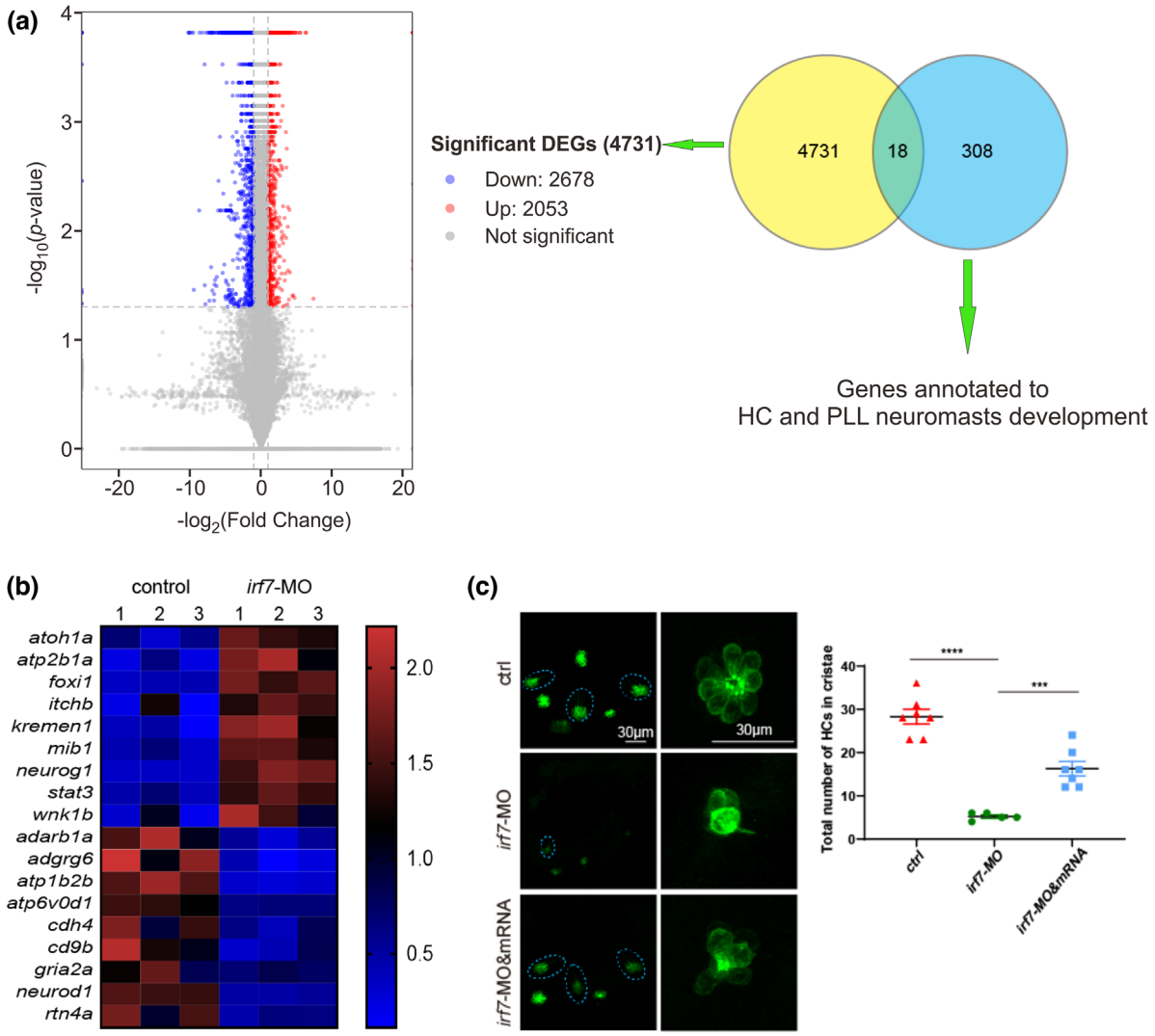

FIGURE 4 Significant differentially expressed genes (DEGs) regulated by *irf7*. (a) Number of DEGs upon knockdown of *irf7*. Among these DEGs, 18 genes are annotated to hair cell (HC) and posterior lateral line (PLL) neuromasts development. (b) Comparison of relative gene expression of the annotated 18 genes between *irf7*-morphants and control. (c) *atp1b2b* mRNA injection rescues crista HC defects induced by *irf7*-knockdown. ***, $P < 0.001$; ****, $P < 0.0001$

and external fertilization and embryo development. Using a zebrafish model for further understanding of how the development of HCs is affected by cellular factors could help to elucidate the mechanisms underlying human hearing loss.

A variety of genes responsible for hearing loss have been identified so far and the majority of them are involved in HC damage (Cunningham & Tucci, 2017; Richardson et al., 2011). Although numerous genes have been identified, it is likely that there are still cohorts of deafness-related genes yet to be discovered. Therefore, identifying such genes is important in elucidating the mechanism of hearing loss and in identifying potential candidates for future gene therapy of deafness.

IRFs are a family of transcription factors that regulate many aspects of innate and adaptive immune responses. The family comprises nine members (IRF1-9) in humans, one of which, IRF7, is mainly functional in the antivirus immune response (Ning et al., 2011). In our study, we found that *irf7* is expressed in the otic vesicle and lateral line in zebrafish larvae, which suggests a potential role for *irf7* in ear development. Although its function in immunology has been scrutinized, the role of *irf7* in the ear, specifically in HC development is still unknown. We used *irf7*-specific morpholino to knockdown the expression of *irf7* to examine the role of *irf7* in zebrafish. Upon downregulation of *irf7*, the number of neuromasts on the lateral line and the number of HCs in the individual neuromast were significantly decreased. We concluded that inhibiting *irf7* expression had a negative effect on neuromast and HC development in zebrafish. In addition, knockdown of *irf7* also hindered embryo development during the early stages, which might result from cytotoxicity in the embryos caused by off-target morpholinos. Not only were the

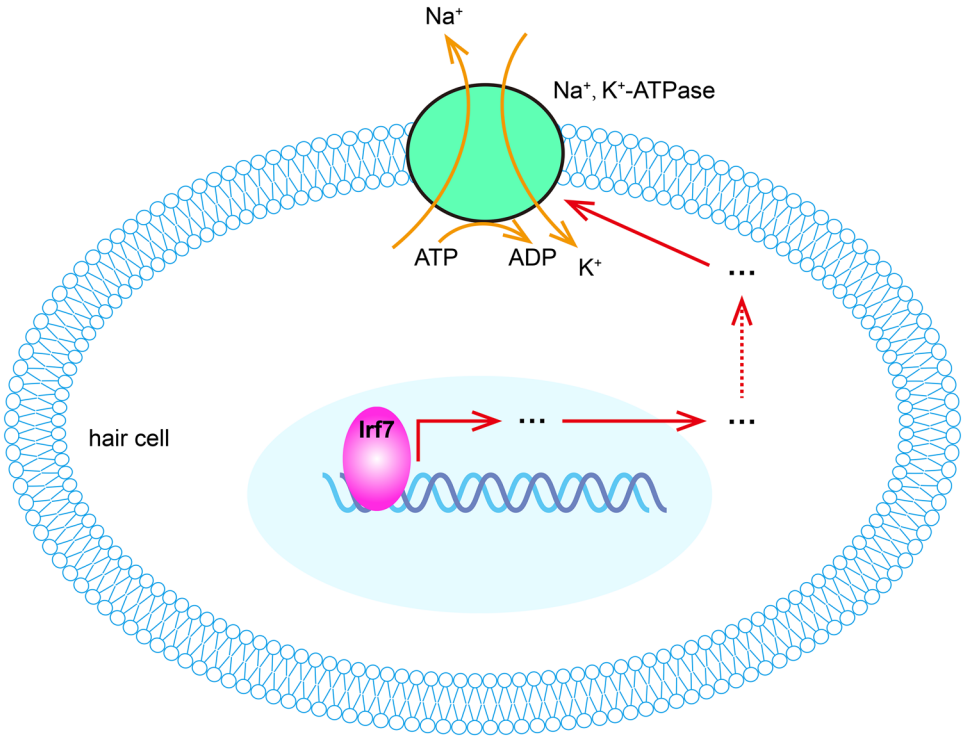

FIGURE 5 Working model of the Irf7 and Atp1b2b in zebrafish hair cells. Irf7, as a transcription factor, can positively regulate the Atp1b2b, which was a component of the Na^+, K^+-ATPase

numbers of HCs reduced in *irf7*-morphants but also the morphology of HCs in the remaining neuromasts became abnormal. The phenotypic changes could be partially rescued by an *irf7* mRNA injection. Hence, a role for *irf7* in regulating the development of HCs in zebrafish was identified. To further investigate whether the loss of HCs in neuromasts resulted from apoptosis (Yang et al., 2018), we used a TUNEL staining kit to label and visualize the apoptotic HCs in *irf7*-morpholino injected *Tg(Brn3c:GFP)* zebrafish embryos. HCs were not labeled in the apoptosis assay, indicating that the decreased number of HCs in *irf7*-morphants was not due to cellular apoptosis. This result provided further confirmation that HC development was influenced by *irf7*. In addition, RNA-seq experiments revealed that 18 altered genes are annotated to HC and PLL development through gene ontology (GO) analysis. Among them, *atp1b2b* was selected to further examine whether it could regulate HC or PLL development downstream of *irf7*. Coinjection of *irf7* morpholino as well as *atp1b2b* mRNA could partially rescue the defective phenotype caused by *irf7* deficiency. It was also consistent with a previous report that *atp1b2b* was associated with neuromast HC development (Wang et al., 2008). However, downregulation of *atp1b2b* gene did not alter the *irf7* or *eya4* mRNA level, indicating the Atp1b2b function downstream of the Irf7 and Eya4. Therefore, we present a working model that Irf7, served as a transcription factor, can positively regulate the *atp1b2b* gene expression, and the Atp1b2b, one of the subunits of the Na^+, K^+-ATPase, participates in the transmembrane transport of Na^+ and K^+ in zebrafish hair cells, which further controls the ion homeostasis and cell survival.

Despite the fact that zebrafish belong to the lower vertebrates, they still share approximately 70% of their genes with humans and more than 80% of the identified genes involved in human disease have a zebrafish counterpart (Das & Rajanikant, 2014). Our current study proposed a novel role of *irf7* in zebrafish HC development, as distinct from the role of *irf7* in immunity. We propose *irf7* for inclusion in the deafness gene library and for further investigation in future clinical studies.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Conceptualization, methodology, investigation, formal analysis and writing of the manuscript: Song-Qun Hu. *Conceptualization, investigation and data curation*: Hui-Min Xu. *Investigation, validation and writing the draft of the manuscript*: Fu-Ping Qian, Chang-Sheng Chen and Xin Wang.

Conceptualization, formal analysis and critical revision of the manuscript: Dong Liu and Lei Cheng. All authors reviewed and approved the final version of the submitted manuscript.

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