High-throughput transcriptome sequencing reveals the key stages of cardiovascular development in zebrafish embryos

Chune Zhou1, Wei Zhao1, Shuqiang Zhang1, Junguo Ma1, Yousef Sultan2 and Xiaoyu Li1*

Abstract

Background: The cardiovascular developmental process is a tightly regulated network involving multiple genes. The current understanding of the molecular mechanism behind cardiovascular development is insufficient and requires further research.

Results: Transcriptome sequencing of three developmental stages in zebrafish embryos was performed and revealed three key cardiovascular developmental stages. Then, the differentially expressed genes (DEGs) involved in cardiovascular development were screened out. The three developmental stages were 18 (T1), 24 (T2), and 42 h post fertilization (hpf) (T3), and the three stages were confirmed by detecting differences in expression between cardiomyocyte and endothelial marker genes (cmlc2, fli1) using in situ hybridization, which represents the characteristics of cardiovascular development. Thousands of DEGs were identified using transcriptome analysis. Of them, 2605 DEGs were in T1-vs-T2, including 2003 up-regulated and 602 down-regulated genes, 6446 DEGs were in T1-vs-T3, consisting of 4608 up-regulated and 1838 down-regulated genes, and 3275 DEGs were in T2-vs-T3, including 2420 up-regulated and 855 down-regulated genes. There were 644 common DEGs and 167 common five-fold higher differentially expressed genes (HDEGs) identified, and Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses were performed using the Database for Annotation, Visualization and Integrated Discovery (DAVID). Significant differences was observed in the levels of gene expression among different developmental stages in multiple GO terms and KEGG pathways, such as cell migration to the midline involved in heart development, cardiovascular system development, circulatory system process for biological processes of GO terms; and cardiac muscle contraction, adrenergic signaling in cardiomyocytes for KEGG pathways. These results demonstrated that these three stages were important period for the development of the cardiovascular system. Lastly, we used quantitative real-time PCR (qPCR) to validate the reliability of RNA-sequencing by selecting 21 DEGs.

Conclusions: These results demonstrated that these three stages represented the important periods for cardiovascular system development of zebrafish and some candidate genes was obtained and provided a solid foundation for additional functional studies of the DEGs.

Keywords: Transcriptome analysis, Cardiovascular system, Zebrafish embryos, Differentially expressed genes

© The Author(s) 2022. Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated in a credit line to the data.
is a dynamic, spatiotemporally, and transcriptionally regulated process involving thousands of genes [3].

Zebrafish are increasingly popular in many fields of scientific research due to their rapid growth, relatively short reproductive period, and their ease of genetic manipulation [4]. They are a particularly good model animal for studying vascular development since they have transparent embryos, can be manipulated genetically [5], and their embryos can survive without blood circulation for approximately seven days post-fertilization. Therefore, it is easy to screen for vascular mutants.

In the present study, we focused on investigating important cardiovascular system-related genes during early embryogenesis, based on the zebrafish developmental model. The differentially-expressed genes (DEGs) among three key developmental stages (18, 24, and 42hpf) were analyzed using integrated transcriptome sequencing. The results demonstrated that these three stages were important for cardiovascular system development. This study provided a preliminary basis for additional exploration of DEG functions, particularly common DEGs.

Results

Characteristics of cardiovascular development at three developmental stages

Three key developmental stages were selected, including 18, 24, and 42hpf (Fig. 1). The characteristics of cardiovascular development at three stages were demonstrated through in situ hybridization using the antisense probe of cardiomyocyte and endothelial marker genes (cmlc2 and fli1 respectively, the probe primers was listed in Supplementary Table 2). A pair of primordia were generated at 18 hpf, one on each side of the midline (Fig. 1a). The primordia on both sides fused to form a single linear heart tube at 24 hpf (Fig. 1b); this is when the heart begins to contract and blood starts to flow. By the time the embryo reaches 42 hpf, the heart tube undergoes looping morphogenesis, the heart chambers are visible, and the ventricle and atrium have been separated (Fig. 1c). The expression of endothelial marker genes (fli1) was only found in the main vessels at 18 hpf and 24 hpf embryos (Fig. 1d, e). However, when the embryo developed to 42 hpf, it was found in intersegmental vessels (ISVs), which connect the dorsal arteries and veins (Fig. 1f) and ensure that blood flows throughout the body. These results revealed that these three stages are so important for the development of the cardiovascular system that some genes of the cardiovascular system developmental network are likely active. Therefore, comparative transcriptome analysis across three stages can provide a foundation for further research into the molecular mechanisms of genes associated with specific biological processes.
an Illumina HiSeq2500 system. The percentage of bases of Q20 ≥ 20 reached 99% (Fig. 2c). The correlation coefficient between the two replicates exceeded 95% (Fig. 2a), demonstrating sufficient repeatability between the two parallel experiments. The 80%-100% gene coverage accounted for approximately 70% of total genes (Fig. 2b). These results demonstrate that the sequencing data was credible enough for further analysis.

Identification of DEGs among three key developmental stages

Thousands of DEGs were found to be up-regulated or down-regulated according to the result of transcriptome analysis across three different developmental stages. (Supplementary table 3, 4, 5). There were 2605 DEGs in T1-vs-T2, including 2003 up-regulated genes and 602 down-regulated genes, 3275 in T2-vs-T3, including 2420 up-regulated genes, and 855 down-regulated genes, and 6446 in T1-vs-T3, including 4608 up-regulated genes and 1838 down-regulated genes (Fig. 3a). T1-vs-T3 had the most DEGs compared to T1-vs-T2 and T2-vs-T3, while T1-vs-T2 had the fewest. Interestingly, there were more up-regulated DEGs than down-regulated DEGs in each comparative group. The number of DEGs up-regulated in T1-vs-T2 was 3.3 times higher than the number of down-regulated ones, 2.8 times higher in T2-vs-T3, and 2.5 times higher in T1-vs-T3. Volcano plots indicated that the number of up-regulated DEGs was significantly higher than the number of down-regulated DEGs (Fig. 3).

Fig. 2 Quality analysis of transcriptome sequencing. a Correlation coefficient maps between different sequencing repeats. b Statistical maps of gene coverage. c Base composition and quality distributions
To further understand DEGs function during three different developmental stages, all DEGs were subjected to GO term analysis and KEGG pathway enrichment analysis [6] (Fig. 4). GO term analysis demonstrated that DEGs in T1-VS-T2 were primarily enriched in some biological processes (p < 0.05), two of them were cell migration to the midline involved in heart development (GO 0,003,318), cardioblast migration to the midline involved in heart field formation (GO 0,060,975) (Fig. 4a, Supplementary table 6). The results indicated that a pair of primordia on both sides migrated to the midline and fused to form an intermediate cone when embryos developed from 18 to 24hpf, which were consistent with the results of in situ hybridization of cardiomyocyte marker gene cmlc2 (Fig. 1). Three of GO terms which enriched in T1-VS-T3 were circulatory system process (GO 0,003,013), cardiovascular system development (GO 0,072,358), and circulation system development (GO 0,072,359) (Fig. 4c, Supplementary table 7). This results indicated that the stages from 18 to 42hpf may be very important for the cardiovascular system development. Four terms enriched in biological processes in T2-VS-T3 were cardiac muscle tissue development (GO 0,051,179), cardiac cell development (GO 0,007,626), cardiac muscle cell development (GO 0,055,006) and cardiac chamber formation (GO 0,003,008) (Fig. 4e, Supplementary table 8). This results demonstrated that the cardiac muscle was developed...
Fig. 4 (See legend on previous page.)
Identification of common DEGs in three stages

To further understand the DEG function in the three stages, common DEGs and common HDEGs of the three comparative groups were identified (Fig. 5a, b). There were 644 common DEGs (Fig. 5b); of them, 568 genes were up-regulated and 76 genes were down-regulated. There were 167 common HDEGs (Fig. 5a), including 161 up-regulated genes and 6 down-regulated genes. Interestingly, hierarchical clustering analysis of the DEGs based on normalized FPKM values indicated that common DEGs (Fig. 5c) and common HDEGs (Fig. 5d) were primarily up-regulated, while only a few were down-regulated. Additionally, differences in T1 vs T2 were less important than differences in T1 vs T3 and T2 vs T3. To further explore the role of DEGs in the three comparative groups, we performed KEGG pathway enrichment analysis on 644 common DEGs and 167 common HDEGs. The results of GO analysis demonstrated that 644 common DEGs were enriched in some terms related to heart development, such as heart process, cardiovascular system development, circulatory system development, circulatory system process (Fig. 5e, Supplementary table 12), while 167 common HDEGs in cardiac myofibril assembly, cardiac cell development, cardiac muscle cell development, heart process and cardiac muscle cell differentiation (Fig. 5g, Supplementary table 13). The results of KEGG enrichment analysis demonstrated both 644 common DEGs and 167 HDEGs were enriched the cardiovascular-related pathways, such as cardiac muscle contraction, adrenergic signaling in cardiomyocytes and PI3K-AKT signaling pathways (Fig. 5f, h, Supplementary table 14, 15). In conclusion, these three developmental stages were possibly crucial for cardiovascular development.

Identification of cardiovascular-related DEGs

Expression profiles demonstrated that common DEGs were associated with multiple pathways. While common DEGs and HDEGs were most significantly enriched in the circulatory system, they were also involved in other pathways, such as protein digestion and absorption and adrenergic signal transduction in cardiomyocytes. Therefore, we selected the genes enriched in the circulatory system and cardiovascular disease for hierarchical cluster analysis (Fig. S1), and the results showed that there were 42 DEGs listed in Table 1 and 11 HDEGs listed in Table 2.

qPCR validation

To validate the reliability of the RNA sequencing data, we selected 21 DEGs (cacnb1sa, acctc1c, cacnb2, cu1633479, symhc3, slc8a1a, myh7, atp2, tnn2d, ryr2b, desma, itgb1b2, lama2, tnn1b, tnn2c, tnn1a, myh7l, myl10, tnn2c, symhc2, ngs), which were mainly related to cardiovascular development and cardiovascular disease pathways. The qPCR results demonstrated that the gene expression levels of these DEGs were similar to the RNA sequencing results, which confirmed the reliability of transcriptome analysis across three key cardiovascular stages (Fig. 6).

Characterization of DEGs expression in different tissues

To further investigate the potential function of certain genes during cardiovascular development, their expression in different tissues was characterized. Most of them were highly expressed in the heart or muscle. For example, the expression level of cacnb4 was highest in the brain, followed by the eyes; the expression of Slc8a1a was highest in the heart, followed by the eyes; the expression of desma was highest in the gills, followed by the heart; the expression of lama2 was highest in the muscles, followed by the heart; the expression of ryr2b was highest in the heart, followed by the muscles and eyes; and the expression of has2 was highest in the muscles, followed by the eyes and the heart (Fig. 7). The results suggest that these genes likely play a key role in cardiovascular development.

Analysis of the spatial expression of DEGs

According to the results of qPCR verification in three stages, most genes showed an increasing trend across the three stages. The results of expression in different tissues found that ryr2b, lama2, slc8a1a, has2, and desma had higher levels of expression in the muscle or the heart than in other tissues. According to the annotation of the ZFIN database, ctnn2 was weakly expressed in the intersegmental vessels, so we chose these 6 genes to detect the spatial expression by the whole embryo in situ hybridization. The results demonstrated that ctnn2 expressed weakly in intersegmental vessels and strongly expressed in nerve tissues (Fig. S3). Desma was expressed in myotomes, hapaxial muscles, pectoral fin muscles, head muscles,
and the heart (Fig. S2). *Has2* was mainly expressed in the heart, posterior somites, and tail bud (Fig. S4). *Lama2* was mainly expressed in the somites (Fig. S5), *Ryr2b* was mainly expressed in the heart (Fig. S6), and *Slc8a1a* was mainly expressed in the heart (Fig. S7).

**Discussion**

**Transcriptome analysis in zebrafish embryogenesis**

The rapid development of high throughput sequencing technology has allowed us to better understand the mechanism of various biological processes, including those of zebrafish. Previous transcriptome analysis primarily focused on early developmental stages [4, 7–9]. Results demonstrated that stage-specific genes are typically preferentially expressed during these stages and that these genes could be used as molecular markers to distinguish one stage from another [9]. This indicates that stage-preferential genes could have close relationships with biological processes. Since the morphological changes of the heart and blood vessels are significant at 18 hpf, 24 hpf, and 42 hpf in zebrafish embryos, the genes preferentially expressed during these three stages must be related to the development of heart and blood vessels. The aim of this study was to screen out genes related to cardiovascular development through transcriptome sequencing of these three stages.

**Important genes involved in cardiovascular development were identified**

Transcriptome analysis of three developmental stages allowed us to screen out 42 genes that could be involved in cardiovascular development. Of these genes, *atp1a2a* regulates zebrafish right-left patterning [10] and plays a key role in establishing proper cardiac laterality in zebrafish [11]. Additionally, certain genes screened through the transcriptome analysis are involved in the cardiovascular developmental process, including cardiac contractility [11–14], cardiomyocyte differentiation [14], vascular stability [15], hematopoiesis [16], PAAs (The pharyngeal arch arteries) development [17] and heart development [18]. Some genes are also expressed in the heart [17, 19], but their functions are largely unknown and require additional study.

In this study, we used whole-mount in situ hybridization to identify the expression of 6 genes in zebrafish embryos. Of these, *desmin* was expressed in the head muscles, trunk muscles, pectoral fin muscles, in developing hearts and fins. These results are consistent with previous reports [20, 21]. *Desmin* has a muscle-specific expression pattern, and is expressed in smooth muscles, skeletal muscles, and myocardium [20]. However, its function in developing hearts is still unclear and requires further research. We found that *cntn2* was mainly primarily expressed in neurons and weakly expressed in intersegmental blood vessels. These results are consistent with those of previous studies [21]. Other research has reported that *cntn2* is involved in branchiomotor neuron migration [22–24], spinal cord regeneration [25], and axon fasciculation [26]. Nevertheless, the role of *cntn2* in intersegmental blood vessels has yet not been reported. Since it is expressed in intersegmental blood vessels, it could play a role in the development of intersegmental blood vessels, but this requires additional study. We determined that *lama2* was primarily expressed in the trunk muscles, which was consistent with previous studies [27, 28]. *Laminins* are the main components of basement members and are required for the development of embryonic and adult tissues [29]. It consists of three chains (alpha, beta, and gamma) which are encoded by different genes. They have different tissue-specific expression patterns and have different functions, which are described in previous reports [28]. Existing reports have demonstrated that *laminins* plays several roles in adults and embryos, including blood vessel formation [30], notochord formation [31], neuron migration [32], and neurogenesis [33, 34]. Interestingly, *laminin* alpha chains have overlapping roles in the formation of notochords and blood vessels [30]. However, little is known about the role of *lama2* in blood vessel formation. Therefore, this requires further study. In addition, our results also showed that *rry2b* was dominantly expressed in the heart, which is consistent with previous studies [35]. However, its function in the heart has not yet been reported, and further research is necessary. Additionally, results of the heart-specific expression patterns of two genes detected in this study (*slc8a1a* and *has2*) was consistent with previous reports in which *slc8a1a* was reported to be required for proper cardiac morphogenesis, while *Has2* plays a key role in vasculature formation [36].

(See figure on next page.)

**Fig. 5** Common DEGs in three stages of zebrafish embryo development. a Common HDEGs were obtained in three stages. b Common DEGs were obtained in three stages. c Hierarchical clustering of 644 common DEGs. d Hierarchical clustering of 167 common HDEGs. e GO enrichment analysis of 644 common DEGs. f KEGG enrichment analysis of 644 common DEGs. g GO enrichment analysis of 167 common HDEGs. h KEGG enrichment analysis of 167 HDEGs.
Fig. 5 (See legend on previous page.)
In conclusion, we screened out the DEGs from three key developmental stages of zebrafish embryonic cardiovascular system using transcriptome analysis, some genes, including \textit{slc8a1a} and \textit{has2}, were previously reported to be involved in cardiovascular development. Some genes were expressed in cardiovascular development, but their function is unknown and need more study in the future. To this end, our research provides more candidate genes for future work and to better understand the role of these genes in embryonic cardiovascular development.

\textbf{Methods}

\textbf{Animal husbandry and embryo collection}
Zebrafish (\textit{Danio rerio}) were purchased from the Institute of Hydrobiology, Chinese Academy of Science (Wuhan, China). Wild-type (AB line) zebrafish were bred and maintained according to standard procedures \cite{37} and staged as described \cite{38}. All animal processing was approved by the Institutional Animal Care and Use Committee of Henan Normal University.

\textbf{RNA extraction, library construction, and sequencing}
Total RNA was isolated using a Trizol reagent kit (Cwbiotech, Beijing, China) according to the manufacturer’s instructions. The resulting RNA was further sequenced using Illumina HiSeq2500 by Gene Denovo Biotechnology Co. (Guangzhou, China).

\textbf{Data filtering and de novo assembly}
Raw reads containing adapters or low-quality bases produced from the sequencing machines were first filtered using fastp \cite{39}. Reads mapped to the ribosome RNA (rRNA) database using the short reads alignment tool Bowtie2 \cite{40} (version 2.2.8) were removed. The remaining reads were mapped to the reference genome using HISAT2. 2.4 \cite{41}. The mapped reads from each sample were assembled using StringTie v1.3.1 \cite{42}.

\begin{table}[h]
\centering
\caption{The cardiovascular development and disease-related genes in common DEGs}
\begin{tabular}{|l|c|c|}
\hline
No & Gene ID & Gene symbol \\
\hline
1 & ENSDARG00000001870 & atp1a1a \\
2 & ENSDARG00000004931 & slc8a3 \\
3 & ENSDARG00000008772 & cacng1a \\
4 & ENSDARG00000010472 & atp1a2a \\
5 & ENSDARG00000013755 & acnt3a \\
6 & ENSDARG00000014804 & cacna2d1a \\
7 & ENSDARG00000018259 & atp1a3a \\
8 & ENSDARG00000019367 & tgbf3 \\
9 & ENSDARG00000020574 & atp2a1 \\
10 & ENSDARG00000020610 & tnt2a \\
11 & ENSDARG00000024141 & cav3 \\
12 & ENSDARG00000029764 & mef2ca \\
13 & ENSDARG00000032242 & tnt2c \\
14 & ENSDARG00000032976 & cmcl1 \\
15 & ENSDARG00000039173 & ctslb \\
16 & ENSDARG00000039832 & gja1 \\
17 & ENSDARG00000041799 & myl13 \\
18 & ENSDARG00000042245 & gdst1 \\
19 & ENSDARG00000042428 & cacna1s \\
20 & ENSDARG00000042552 & zgc172,323 \\
21 & ENSDARG00000053201 & pepc2r5b \\
22 & ENSDARG00000054931 & desma \\
23 & ENSDARG00000058656 & myl10 \\
24 & ENSDARG00000062592 & zgc158,296 \\
25 & ENSDARG00000070923 & cdh5 \\
26 & ENSDARG00000075549 & rapge4 \\
27 & ENSDARG00000075924 & atp1a2a \\
28 & ENSDARG00000076833 & atp2a1 \\
29 & ENSDARG00000079564 & myh7 \\
30 & ENSDARG00000087574 & nox1 \\
31 & ENSDARG00000089806 & siderkey-239j18.3 \\
32 & ENSDARG00000103747 & cav1 \\
33 & ENSDARG00000103969 & smhc2 \\
34 & ENSDARG00000114031 & myh7 \\
35 & ENSDARG00000114818 &CU633479.5 \\
36 & ENSDARG00000115657 & pRca \\
37 & ENSDARG00000116713 & sich73-265d7.2 \\
38 & MSTRG.11363 & itgb1 \\
39 & MSTRG.1910 & mef2c \\
40 & MSTRG.25366 & ppp3r3b \\
41 & MSTRG.7667 & acta1 \\
42 & MSTRG.8268 & ctstv \\
\hline
\end{tabular}
\end{table}

\begin{table}[h]
\centering
\caption{The cardiovascular development and disease-related genes in common HDEGs}
\begin{tabular}{|l|c|c|}
\hline
No & Gene ID & Gene symbol \\
\hline
1 & ENSDARG00000008772 & cacng1 \\
2 & ENSDARG00000010472 & atp1a2a \\
3 & ENSDARG00000018259 & atp1a3a \\
4 & ENSDARG00000020574 & atp2a1 \\
5 & ENSDARG00000032242 & tnt2c \\
6 & ENSDARG00000032976 & cmcl1 \\
7 & ENSDARG00000042245 & myl13 \\
8 & ENSDARG00000042552 & cacna1s \\
9 & ENSDARG00000076833 & atp2a1b \\
10 & ENSDARG00000079564 & myh7 \\
11 & MSTRG.7667 & acta1 \\
\hline
\end{tabular}
\end{table}
DEGs analysis and function annotation

DEGs analysis was performed by DESeq2 [43] software across the three stages. Genes with false discovery rate (FDR) ≤ 0.05 and absolute fold change ≥ 2 were considered as DEGs. To analyze DEGs function among the 18, 24, and 42 hpf stages, all DEGs were mapped to terms in the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (http://www.genome.jp/kegg/pathway.html) and the Gene Ontology database (http://www.geneontology.org/). GO terms and KEGG pathways with FDR < 0.05 were deemed as significantly enriched GO terms and KEGG terms.

Quantitative real-time PCR (qRT-PCR) validation

To validate the reliability of the transcriptome sequencing, qPCR was performed for 21 DEGs. The primers were designed using Primer 5.0 and sequences were provided in Supplementary Table 1. Total RNA was extracted from 50 embryos or tissue from two adult zebrafish. First strand cDNA was reversely transcribed from 1 μg RNA

Fig. 6  Validation of DEGs by qPCR. The expression of 18 hpf was set as 1, and the relative expression level of the other two stages (24 hpf and 42 hpf) was calculated for several genes. The asterisk indicates significantly different expression levels (* P < 0.05; **, *** and **** P < 0.01)
with HIFIScript 1st strand cDNA synthesis kit (Cwbio- 
lich, China). The independent experiment was performed 
in triplicate. The rpl13a gene was used as the internal 
control. The reaction system (10 μl) comprised 0.2 μl 
cDNA, 5 μl SYBR green master mix, 0.4 μl each primer 
(20 μM), and 4 μl nuclease-free water. The PCR conditions 
were as follows: 10 min at 95 °C, followed by 45 cycles at 
95 °C for 15 s, 60 °C for 60 s, and a cooling stage at 4 °C. 
The \(2^{-\Delta\Delta C_{\text{t}}} \) method was used to analyze expression levels.

Expression profile of DEGs in different tissues
To characterize the expression profile of DEGs in different 
tissues, qPCR was performed and used eight tissues 
from adult zebrafish as templates. The reaction system 
and PCR conditions were the same as those above.

Spatial expression of DEGs
To further verify their spatial expression, six genes likely 
involved in cardiovascular development were selected to 
determine their expression sites in embryo development 
via whole-mount in situ hybridization. The primers of 
the probe were presented in Supplementary Table 2. 
The PCR fragments were cloned into a pGEM-T easy 
vector and sequenced to verify whether the fragment 
was ligated to the vector. The antisense and sense 
probe were transcripted by SP6 or T7 to generate the 
DIG-labeled probe using the linearized vector, includ-
ing PCR target fragments as a template. Whole-mount 
in situ hybridization was performed as previously 
described [44].

Statistical analysis
SPSS 20.0 was used for statistical analysis. Data was pre-
sented as mean ± SEM. Difference between groups we 
analyzed using the Student’s t-test. \( P \) values < 0.05 were 
considered to be statistically significant.

Abbreviations
DEGs: Differentially expressed genes; GO: Gene ontology; KEGG: Kyoto ency-
clopedia of genes; qPCR: Quantitative real-time polymerase chain reaction.
Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12864-022-08808-x.

Additional file 1: Fig. S1. The genes involving in cardiovascular development screened from the common DEGs and HDEGs. Fig. S2. Spatial expression of desma by whole mount embryo in situ hybridization. Fig. S3. Spatial expression of cnnt2 by whole mount embryo in situ hybridization. Fig. S4. Spatial expression of ha2 by whole mount embryo in situ hybridization. Fig. S5. Spatial expression of lam2 by whole mount embryo in situ hybridization. Fig. S6. Spatial expression of ry2b by whole mount embryo in situ hybridization. Fig. S7. Spatial expression of slc8a1a by whole mount embryo in situ hybridization.

Additional file 2: Table S1. The primers of validated genes.

Additional file 3: Table S2. The probe primers of genes.

Additional file 4: Table S3. The DEGs of T1 VS T2.

Additional file 5: Table S4. The DEGs of T1 VS T3.

Additional file 6: Table S5. The DEGs of T2 VS T3.

Additional file 7: Table S6. The enriched GO terms in DEGs of T1 vs T2.

Additional file 8: Table S7. The enriched GO terms in DEGs of T1 vs T3.

Additional file 9: Table S8. The enriched GO terms in DEGs of T2 vs T3.

Additional file 10: Table S9. The enriched KEGG pathways in DEGs of T1 vs T2.

Additional file 11: Table S10. The enriched KEGG pathways in DEGs of T1 vs T3.

Additional file 12: Table S11. The enriched KEGG pathways in DEGs of T2 VS T3.

Additional file 13: Table S12. The enriched GO terms in 644 common DEGs.

Additional file 14: Table S13. The enriched GO terms in 167 common HDEGs.

Additional file 15: Table S14. The enriched KEGG pathways in 644 common DEGs.

Additional file 16: Table S15. The enriched KEGG pathways in 167 common HDEGs.

Acknowledgements

Not applicable.

Code availability

Not applicable.

Authors’ contributions

C. Zhou conceived the research and performed all experiments in this study. W. Zhao provided some assistance in collecting samples. S. Zhang provided valuable technical advice. J. Ma gave technical assistance. Yousef Sultan provide assistance in language grammar checking. X. Li provided research instruction throughout the study and reviewed the manuscript. The author(s) read and approved the final manuscript.

Funding

This research was supported by the Key Research Project of Henan University in China (Grant No.19zx011).

Availability of data and materials

RNA-seq data sets in our study are available in the National Centerfor Biotechnology Information Gene Expression Omnibus under the accession number GSE207476.

Declarations

Ethics approval and consent to participate

Fish were handled in accordance with guidelines in China Law for Animal Health Protection and Instructions for Granting Permits for Animal Experimentation for Scientific Purposes (Ethics approval No. SCXK (YU) 2003–0001). All methods are reported in accordance with ARRIVE guidelines for the reporting of animal experiments. All experiments were approved by the Institutional Animal Care and Use Committee of Henan Normal University.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

1 College of Life Science, Henan Normal University, Xinxiang 453007, Henan, China. 2 Department of Food Toxicology and Contaminants, National Research Centre, Dokki, Cairo 12622, Egypt.

Received: 8 April 2022 Accepted: 25 July 2022

Published online: 13 August 2022

References

1. Fishman M, Chien K. Fashioning the vertebrate heart: earliest embryonic decisions. Development. 1997;124:2099–177.
2. Edward M. Conway DC, Peter Carmeliet. Molecular Mechanisms of Blood Vessel Growth. Cardiovasc Res. 2001;49(3):507–21.
3. Kathiriya IS, Nora EP, Brunneau BG. Investigating the transcriptional control of cardiovascular development. Circ Res. 2015,110:700–14.
4. Vesterlund L, Jiao H, Unneberg P, Hovatta O, Kere J. The zebrafish transcriptome during early development. BMC Dev Biol. 2011;11:30.
5. Bradbury J. Small fish, big science. PLoS Biol. 2004;2(5):E148.
6. Kanehisa M, Goto S. KEGG: Kyoto Encyclopedia of Genes and Genomes. Nucleic Acids Res. 2000;28:287–30.
7. Aanes H, Collas P, Aleström P. Transcriptome dynamics and diversity in the early zebrafish embryo. Brief Funct Genomics. 2014;13(2):95–105.
8. Aanes H, Winata CL, Lin CH, Chen JR, Srinivasan KG, Lee SG, Lim AT, Hajan HS, Collas P, Bourque G, et al. Zebrafish mRNA sequencing deciphers novelties in transcriptome dynamics during maternal to zygotic transition. Genome Res. 2011;21(8):1328–38.
9. Yang H, Zhou Y, Gu J, Xie S, Xu Y, Zhu G, Wang L, Huang J, Ma H, Yao J. Deep mRNA sequencing analysis to capture the transcriptome landscape of zebrafish embryos and larvae. PLoS ONE. 2013;8(5):e64058.
10. Shu X, Huang J, Dong Y, Choi J, Langenbacher A, Chen JN. Na, K-ATPase alpha2 and Ncx4a regulate zebrafish left-right patterning. Development. 2007;134(10):1921–30.
11. Shu X, Cheng K, Patel N, Chen F, Joseph E, Tsai HJ, Chen JN. Na, K-ATPase is essential for embryonic heart development in the zebrafish. Development. 2003;130(25):6165–73.
12. Sehnert AJ, Huq A, Weinstein BM, Walker C, Fishman M, Stainier DY. Cardiac troponin T is essential in sarcomere assembly and cardiac contractility. Nat Genet. 2002;31(1):106–10.
13. Chen Z, Huang W, Dahme T, Rottbauer W, Ackerman MJ, Xu X. Depletion of zebrafish essential and regulatory myosin light chains reduces cardiac function through distinct mechanisms. Cardiovasc Res. 2008;79(1):97–108.
14. Meder B, Lauffer C, Hassel D, Just S, Marquart S, Vogel B, Hess A, Fishman MC, Katus HA, Rottbauer W. A single seerine in the carboxyl terminus of cardiac essential myosin light chain-1 controls cardiomyocyte contractility in vivo. Circ Res. 2009;104(5):650–9.
15. Montero-Balaguer M, Swirsding K, Osengo F, Cotelli F, Mione M, Dejana E. Stable vascular connections and remodeling require full expression of VE-cadherin in zebrafish embryos. PLoS ONE. 2009;4(8):e5772.

16. Jiang Q, Liu D, Sun S, Hu J, Tan L, Wang Y, Gui Y, Yu M, Song H. Critical role of connexin43 in zebrafish late primitive and definitive hematopoiesis. Fish Physiol Biochem. 2010;36(4):945–51.

17. Abrial M, Paffett-Luagassy N, Jeffrey S, Jordan D, O’Loughlin E, Frederick C, 3rd, Burns CG, Burns CE. TGF-β signaling is necessary and sufficient for pharyngeal arch artery angioblast formation. Cell Rep. 2017;20(4):973–83.

18. Razaghi B, Steele SL, Prynkozhij SV, Stoyek MR, Hill JA, Cooper MD, McDonald L, Lin W, Daugaard M, Crapoulet N, et al. Hac1 influences zebrafish cardiac development via ROS-dependent mechanisms. Dev Dyn. 2012;241(2):289–303.

19. Haverinen J, Hassinen M, Dash SN, Vornanen M. Expression of calcium channel transcripts in the zebrafish heart: dominance of T-type channels. J Exp Biol. 2018;221(Pt 10):179226.

20. Loh SH, Chan WT, Gong Z, Lim TM, Chua KL. Characterization of a zebrafish (Danio rerio) desmin cDNA: an early molecular marker of myogenesis. Differentiation. 2000;65(5):247–54.

21. Thisse B, Wright GJ, Thisse C. Embryonic and Larval Expression Patterns from a Large Scale Screening for Novel Low Affinity Extracellular Protein Interactions. ZFIN Direct Data Submission. 2008.

22. Sittaramane V, Sawant A, Wolman MA, Maves L, Halloran MC, Chandrasekhar A. The cell adhesion molecule Tag1, transmembrane protein Sibtm/Vangl2, and Laminin1 exhibit genetic interactions during migration of facial branchiomotor neurons in zebrafish. Dev Biol. 2009;325(2):363–73.

23. Stockinger P, Maître JL, Heisenberg CP. Defective neuroepithelial cell cohesion affects tangential branchiomotor neuron migration in the zebrafish neural tube. Development. 2011;138(21):4673–83.

24. Lang DM, Warren JT, Kisa C, Stuemer CAO. Topographic restriction of TAG-1 expression in the developing retinotectal pathway and target dependent reexpression during axon regeneration. Mol cellular neurosci. 2001;17(2):398–414.

25. Hou B, Lin J-F, Fan H-C, Ma L-P, Shen Y-Q, Schachner M. The cell neural adhesion molecule contactin-2 (TAG-1) is beneficial for functional recovery after spinal cord injury in adult zebrafish. PLoS One. 2012;7(12):e52576.

26. Gurung S, Asante E, Hummel D, Williams A, Feldman-Schultz O, Halloran MC, Sittaramane V, Chandrasekhar A. Distinct roles for the cell adhesion molecule Contactin2 in the development and function of neural circuits in zebrafish. Mech Dev. 2018;152:1–12.

27. Hall TE, Bryson-Richardson RJ, Berger S, Jacoby AS, Cole NJ, Hollway GE, Berger J, Currie PD. The zebrafish cadf13 mutant implies extracellular matrix adhesion failure in laminin alpha2–deficient congenital muscular dystrophy. Proc Natl Acad Sci U S A. 2007;104(17):7092–7.

28. Szta T, Berger S, Currie PD, Hall TE. Characterization of the laminin gene family and evolution in zebrafish. Dev Dyn. 2011;240(2):422–31.

29. Zinkevich NS, Bosenko DV, Link BA, Semina EV. Laminin alpha 1 gene family and evolution in zebrafish. Dev Dyn. 2011;240(2):422–31.

30. Pollard SM, Parsons MJ, Kamei M, Kettleborough RN, Thomas KA, Pham VN, Bae MK, Scott A, Weinstein BM, Stemple DL. Essential and overlapping roles for laminin alpha chains in notochord and blood vessel formation. Dev Biol. 2006;291(1):64–76.

31. Parsons MJ, Pollard SM, Salude L, Feldman B, Coutinho P, Hirst EM, Stemple DL. Zebrafish mutants identify an essential role for laminins in notochord formation. Development. 2002;129(13):3137–46.

32. Grant PK, Moens CB. The neuroepithelial basement membrane serves as a boundary and a substrate for neuron migration in the zebrafish hindbrain. Neural Dev. 2010;5:9.

33. Jiang YJ, Brand M, Heisenberg CP, Beuchle D, Furutani-Seiki M, Kelsh RN, Warga RM, Granato M, Haffter P; Hammenschmidt M, et al. Mutations affecting neurogenesis and brain morphology in the zebrafish. Danio rerio Development. 1996;123:205–16.

34. Paulus JD, Halloran MC. Zebrafish bashful/laminin-alpha 1 mutants exhibit multiple axon guidance defects. Dev Dyn. 2006;235(1):213–24.

35. Wu HT, Brennan C, Ashworth R. Ryonodine receptors, a family of intracellular calcium ion channels, are expressed throughout early vertebrate development. BMC Res Notes. 2011;4(1):541.

36. Rost MS, Sunam S, Hyaluronic acid receptor Stabilin-2 regulates Erk phosphorylation and arterial–venous differentiation in zebrafish. eLife. 2014;9(2):e08614.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:
- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

Learn more: biomedcentral.com/submissions