

Protection role of Astragalus polysaccharide against Cardiac injury in tnnt2a mutant Zebrafish model of Dilated Cardiomyopathy

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Research Article

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Abstract

Background

Dilated cardiomyopathy(DCM) is a severe and irreversible heart disease characterized by dilated ventricles and decreased myocardial function. DCM has a poor prognosis and a very low survival rate, with a 5-year mortality rate of 15–50%, and is an important cause of sudden cardiac death and heart failure. Genetic factors play an important role in the pathogenesis of DCM. Mutations in the cardiac troponin T (*tnnt2*) gene represent an important subset of known pathogenic variants that bind to DCM. However, there are currently few specific drugs available to treat DCM caused by these gene mutations. Astragalus polysaccharide (APS), the main active ingredient of *Astragalus mongholicus* Bunge(Huangqi), is widely used in China to treat cardiovascular diseases, including DCM. This study explored drugs for the treatment of DCM caused by *tnnt2a* mutation and found the protective effect of Astragalus polysaccharide on *tnnt2a*-mutant dilated cardiomyopathy.

Methods

The *tnnt2a*^{-/-} mutant zebrafish was used as a DCM model to compare with the APS-administered group. Counting the survival rate and measuring the sinus venosus-bulbus arteriosus (SV-BA) distance to observe changes in cardiac output. Histopathological changes were observed with hematoxylin and eosin (HE) staining and Tunel staining. Transcriptomes of zebrafish DCM group and APS-treated group were investigated by RNA-seq method. qRT-PCR detection of apoptosis-related genes expression.

Results

We found that APS markedly increased the heart rate and ATP content, significantly inhibited the level of cardiac tissue edema, which are essential for improving survival rate in *tnnt2a*^{-/-} zebrafish. Moreover, APS interfere some key genes related to muscle fibers, such as titin b (*ttnb*) and myomesin 3 (*myom3*), APS intervention significantly participated in Rap1 signaling pathway, PI3K-Akt signaling pathway, Jak-STAT signaling pathway, Wnt signaling pathway. The qRT-PCR results revealed that APS decreased the expression of Bax, Caspase-3, Caspase-9 but increased the expression of Bcl-2 in DCM zebrafish.

Conclusions

Our findings suggest that APS could improve survival rate in Dilated Cardiomyopathy, and has a positive protective effect on the myocardium in the *tnnt2a* mutant zebrafish model of DCM.

1 Introduction

Dilated cardiomyopathy (DCM) is a primary myocardial disease, and the disease condition became progressively worse may result from the ventricular remodeling, the heart expands and the myocardial contraction function decreases^[1]. DCM has a poor survival rate, with a 5-year mortality rate of 15–50%^[2], and remains the leading cause of heart transplantation^[3]. The main clinical manifestations of dilated

cardiomyopathy are refractory heart failure, malignant arrhythmia, thromboembolism, cardiogenic shock, and even sudden death^[4].

Genetic factors play an important role in the pathogenesis of DCM. More than 50 genes related to DCM have been identified^[5]. Genetic screening of some Chinese patients with DCM revealed that the deletion of Cardiac troponin T (*tnnt2*) may be one of the causative factors^[6–7]. As is reported that the variation of TNNT2 is related to the high tendency of life-threatening cardiomyopathy in childhood and youth^[8].

The current models of dilated cardiomyopathy are mostly drug-induced, such as doxorubicin^[9] and furazolidone^[10], which are insufficient to reflect all the causes of dilated cardiomyopathy. Additionally, transgenic mammalian DCM models are often laborious and costly. As a novel cardiovascular disease animal model, zebrafish has good visibility in evaluating drug toxicity, efficacy, and drug screening. Therefore, we used CRISPR/Cas9 technology to obtain *tnnt2a* knockout mutant zebrafish. Homozygous mutant zebrafish (Tg:*tnnt2a*^{-/-}) exhibited phenotypes such as cardiac dilatation and asystole, similar to the clinical symptoms of DCM patients^[11]. The *tnnt2* mutant zebrafish was convenient and predictive for high-throughput in vivo efficacy assessment and screening of DCM therapeutic drugs. Most importantly, *tnnt2a*^{-/-} mutant zebrafish model is an available and original DCM model.

At present, it has been recognized that dilated cardiomyopathy of all ages is inseparable from genetic factors, and the treatment is often lack of specificity^[12]. *Astragalus mongholicus* Bunge (Huangqi) is often used in the treatment of DCM, which can effectively alleviate heart rate and prolong walking distance^[13]. Our previous clinical studies have shown that Kuoxinfang with Huangqi as the main drug can improve the symptoms and cardiac function of DCM patients to a certain extent. In addition, we also found that Kuoxinfang can improve the survival rate of the *tnnt2a* mutant zebrafish model^[14]. The main active components of Huangqi are saponins, flavonoids, and polysaccharides. APS is an important bioactive ingredient extracted from Huangqi^[15], which has the effect of protecting the heart^[16], as well as various other pharmacological activities, such as anti-inflammatory^[17], protecting mitochondria^[18], inhibition of apoptosis^[19].

Therefore, in this experiment, we used *tnnt2a* mutant zebrafish as a model of dilated cardiomyopathy to investigate the cardioprotective effect of APS on DCM.

2 Materials And Methods

Zebrafish husbandry and reproduction

Both the Tg:*tnnt2a*^{+/-} mutant strain and wild-type AB strain were purchased from Fudan University. Zebrafish were reared under standard temperature (28°C) and 14h light:10h dark cycle photoperiod conditions, and were fed with fresh paramecia 3 times a day. Fertilized embryos were collected promptly and placed in an egg-water incubator at 28°C. Embryos at 24h post-fertilization(hpf) were fed with egg

water containing 0.2 mM N-phenylthiourea (Sigma-Aldrich, St. Louis, MO, USA) to suppress skin pigment production, which was beneficial during subsequent visual observations were performed in microscopy imaging experiments. All animal experiments were performed according to European guidelines and in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

DCM model in zebrafish

We have reported previously that *tnnt2a* mutant zebrafish was generated by knocking out the related gene using CRISPR/Cas9 technology^[11]. Moreover, mature F0 zebrafish were crossed with wild-type AB to produce offspring containing heterozygous mutants that were in crossed to obtain homozygous mutants. Genotyping was performed via PCR-sequencing to indicate that *tnnt2a* was deleted as reported.

tnnt2a Genotyping Primer Forward: 5'-gtaagcgcacatggagaaggac-3',

tnnt2a Genotyping Primer Reversed: 5'-gcgacatcacagagccaaat-3'.

DCM zebrafish models were divided into two groups: One group named model 1 was *tnnt2a*^{-/-} generated by crossing with *tnnt2a*^{+/-}, which is similar to the clinical phenotypes of patients with dilated cardiomyopathy, such as cardiac arrest, atrial and ventricular enlargement, and also obvious influence on activity (Figure 1).

Tol2 transposon technology was used to insert the target gene *tnnt2a* into the zebrafish genome, and the Tet-On doxycycline (DOX) system induced the expression of *tnnt2a*^[20]. Treatment with DOX produced higher levels of *tnnt2a* mRNA that were comparable to wild-type hearts. In addition, Tg:*tnnt2a*^{-/-} transgenic zebrafish had persistently enlarged atria and ventricles, persistent abdominal edema, and typically died at 6-7 day-post-fertilization(dpf). Therefore, our study used the Tg:*tnnt2a*^{-/-} transgenic zebrafish model with the heart recovered to beat after the addition of DOX as model 2 (Figure 2).

Drugs and Collocation

APS(LOT:C18M6Y1) was purchased from Shanghai Yuanye Biotechnology (Shanghai, China). Doxycycline(DOX), N-phenylthiourea(PTU), Trizol were purchased from Sigma-Aldrich (St. Louis, MO, United States). HE staining kit, One Step TdT-Mediated dUTP Nick End Labeling(TUNEL) Apoptosis Assay Kit, and 4,6diamidino-2-phenylindole (DAPI) staining solution was purchased from Beyotime Biotechnology (Shanghai, China). APS stock solution was prepared with dimethyl sulfoxide (DMSO) at a concentration of 10 mg/ml, stored at -20 °C, and diluted to 15 µg/ml when used.

Assessment Effects of APS on DCM Zebrafish

Homozygous zebrafish(*tnnt2a*^{-/-}) with DCM phenotype were picked under the microscope after 30 hpf. Zebrafish were placed in a 12-well plate with 10 well-grown zebrafish embryos per well, 30 per group. Preliminary experimental results show that the safe concentration of APS is 15µg/ml. The zebrafish has severe heart malformation with obvious phenotype from 3dpf, both model 1 and model 2 were treated with APS for 48h. The embryos receiving egg medium only served as control. Untreated zebrafish were used to confirm that the vehicle solvent did not cause any side effects on zebrafish. The medium of all treatment groups was refreshed every 24h. Each experiment was repeated at least three times.

Morphological observation of zebrafish

At 48 hpf and 72 hpf, zebrafish were visually tracked from microplates and observed for viability, heart rate, and cardiac dilatation under a fluorescence microscope(Olympus IX81 Motorized Inverted Microscope, Japan)equipped with a digital camera(DP controller, Soft Imaging System, Olympus).

Terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL)

Zebrafish embryos from each group were collected and the hearts were dissected under a microscope. Under light-proof conditions, add 30 40µl TUNEL and DAPI to the zebrafish heart, incubate at 37°for 12h, and the incubation time can be appropriately extended. Then washed with PBST and sealed with small coverslips after gradient addition of 20%, 50%, and 70% glycerol. Analyze the samples under the fluorescence microscope and observe the red fluorescence. Laser confocal scanning of DAPI- stained blue channel and TUNEL-stained red channel.

RNA-seq and Bioinformatics Analysis

The zebrafish transcriptome was sequenced, and the bioinformatics data were analyzed according to the methods of our group's previous studies^[21]. Double-terminal sequencing was performed on the Illumina Hi-2000 genome analysis platform. The terminal RNA sequences were paired and the data processed using the fastq format of the Illumina genome analyzer. Cuffdiff software was used for differential gene analysis. Differentially expressed genes were subjected to GO functional annotation and analysis and KEGG Pathway significant enrichment analysis in the DAVID database.

qRT-PCR

Total RNA was extracted from zebrafish embryos using a TRIzol kit. Next, approximately 1µg total RNA was reverse transcribed to synthesize the cDNA. qRT-PCR was performed using SYBR Green detection reagents in a StepOne Plus PCR machine. The relative mRNA levels were calculated by the $2^{-\Delta\Delta C_t}$ method and displayed as fold changes relative to the control group, and β -actin was used as an endogenous control gene to normalize gene expression. The zebrafish β -actin primers were 5'-TTGACAACGGCTCCGGTATG-3'(F) and 5'-TCCCATGCCAACCATCACTC-3'(R); bcl-2 primers were 5'-TGTGCGTGGAAAGCGTCAA-3'(F) and 5'-GTCTCTCTGCTGACCGTACAT-3'(R); bax primers were 5'-CGGAGATGAGCTGGATGGAA-3'(F) and 5'-CAAGGCGACAGGCAAAGTAG-3'(R); caspase-3 primers were 5'-

CCCAGTGGAGGCAGATTTCC-3'(F) and 5'-GAGCATTGAGACGATGCAGG-3'(R); caspase-9 primers were 5'-TAAATGACCGCAAGGGCTCC-3'(F) and 5'-GCCTCAGTGCCATGTGAAAG-3'(R). Each sample was tested in triplicates.

Statistical Analysis

All results are expressed as means $\pm$ standard deviation (SD). The survival rate was compared by the Cox Mantel test, One way ANOVA was used for comparison between multiple groups, and the LSD-t test was used for comparison between two groups by using GraphPad Prism 6.0 software. Values of $p < 0.05$ were considered to indicate statistical significance.

3 Result

Astragalus polysaccharide improves the survival rate and morphology of DCM zebrafish.

Compared with the zebrafish in the DCM model 1 group, the survival rate of zebrafish after APS treatment was significantly improved. Specifically, part of the zebrafish in the model1 and model2 groups have a marked reduction in the survival rate at 5dpf. In contrast, after APS intervention, the survival rate of zebrafish was increased (Fig. 3A). We investigated whether the rise of the survival rate is consistent with cardio output in DCM zebrafish model systems. We found that the heart rate of the DCM zebrafish in the APS treatment group was significantly increased ($P < 0.01$). (Fig. 3B). APS can increase ATP content (Fig. 3C), thereby building cardiac energy reserves, increasing cardiac output, and thus improving survival rate. After treatment with APS, compared with control group, the embryos showed enlarged heart and tissue edema decreased (Fig. 3D). To confirm the ventricular dilatation of the zebrafish in each group, we measured the distance of the arterial bulbar sinus (SV-BA), the SV-BA of the zebrafish in both model groups increased (Fig. 3E-F). These results indicate that Astragalus polysaccharide lengthen the survival time may be necessary to stronger heart contractions and higher cardio putput.

Astragalus polysaccharide can relieve swelling and cardiac hypertrophy in DCM zebrafish.

We performed HE staining on zebrafish and found that compared with the control group, the model zebrafish had tissue edema, disordered arrangement, enlarged cell space, and significantly larger heart. APS treatment showed that zebrafish cardiomyocytes were more tightly packed and cardiac tissue edema was reduced (Fig. 4).

Astragalus polysaccharide can interfere the transcriptome sequencing in DCM zebrafish

To further understand the molecular mechanism of APS cardio protection, we analyzed changes in gene expression levels in zebrafish after APS treatment by transcriptomic techniques. In addition, AB was used as wild type, tnnt represents model 1, tnnt15 represents 15 μ g/ml APS treatment group 1, DOX represents model 2, and DOX15 represents 15 μ g/ml APS treatment group 2.

Subsequently, using RNA sequencing, we selected DEGs that were up-regulated in the model group compared to the AB control group, and DEGs that were down-regulated in the APS group compared to the model group. We screened 35 and 130 overlapping central genes in *tnnt* model 1 and model 2, respectively (Fig. 5). Further analysis of these identified DEGs revealed that some key genes related to ATP-binding activity, actin filaments, and muscle fibers were significantly affected by APS, such as titin b (*ttnb*), myomesin 3 (*myom3*), fibroblast growth factor 11b (*fgf11b*), forkhead-associated phosphopeptide-binding domain 1 (*fhad1*), signal-induced proliferation-associated 1.

Next, we explored the functional annotation of different genes in cardiomyocytes using Gene Ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis. The DEGs were significantly involved in biological process (GO: BP), including cardiac muscle fiber development, cardiac muscle tissue morphogenesis. The DEGs were contained in cellular component (GO: CC), including mitochondrial outer membrane translocase, myosin. DEGs were included in molecular function (GO: MF), inclusive of modulation of the activity of the ATPase activity, G-protein coupled serotonin receptor activity (Fig. 6A, 6B). Additionally, DEGs of APS intervention significantly participated in dilated cardiomyopathy, MAPK signaling pathway, TGF- β signaling pathway, Rap1 signaling pathway, PI3K-Akt signaling pathway, Jak-STAT signaling pathway, ErbB signaling pathway (Fig. 6C, 6D). In some cases, the related genes and regulated transcription factors involved in Rap1 signaling pathway are *fgf11b*, *ephb2a*, *sipa1*, *rapgef5b*; those associated with MAPK signaling pathway are *sbno2a* and *hsp70*. Our work also showed that *nfk1b* and *fhad1* are implicated in NF- κ B pathway; *chico*, *fosl1*, and *fmnl2* are related with Wnt pathway.

Astragalus polysaccharide can Inhibit Apoptosis in DCM Zebrafish.

We used TUNEL staining to observe the nuclei and apoptosis of dissected zebrafish hearts, and the apoptosis of zebrafish hearts after drug administration. The control group showed that the zebrafish had a normal heart outline, closely packed cells, and fewer apoptotic cells. The atria and ventricles of zebrafish in both model 1 group and model 2 group were significantly enlarged, the number of cells decreased, and the number of apoptotic cells increased. At the same time, the cells in the model group were sparse and the nuclei became larger. After APS intervention, the number of apoptotic cells was less than that in the model group, and the atria and ventricles were still enlarged (Fig. 7).

In addition, APS also inhibited apoptosis and regulated expressions of key apoptotic protein, including B cell lymphoma-2 (Bcl-2) and Bcl-2 Associated X Protein (Bax) and caspase-3 and -9. It is worth noting that the mRNA expression of Bax, Caspase-3, Caspase-9 in APS-treated zebrafish was significantly declined, but the mRNA expression of Bcl-2 increased. (Fig. 8).

4 Discussion

Dilated cardiomyopathy is one of the most common causes of chronic heart failure. The main clinical manifestations of dilated cardiomyopathy are low ejection fraction values, progressive heart failure, malignant arrhythmias, cardiogenic shock and sudden death, which can occur at any stage of disease

progression. Up to now, there is no specific treatment, and the prognosis is very poor, with a 5-year survival rate of less than 50%^[22].

Familial DCM accounts for about 20%-50% of all cases, and more than 50 gene mutations have been confirmed to cause DCM^[23]. Evaluating the genetic and clinical evidence of DCM revealed that mutations in 12 genes, including MYH7, LMNA, BAG3, *tnnt2*, TNNC1, and PLN were significantly enriched in specific patient subgroups. Mutations in these genes may account for the majority of DCM patients^[24]. The sarcomere is the target of mutations in cardiomyopathy. The protein produced by mutant alleles affects the formation and function of sarcomere^[25]. Mutations in the gene encoding cTnT, TNNT2, were significantly correlated with DCM^[26-27]. Genetic screening of some Chinese DCM patients found that the deletion of *tnnt2* may be one of the causes^[28-29]. As is reported that the variation of TNNT2 is related to the high tendency of life-threatening cardiomyopathy^[30].

However, there is no *Tnnt2a* mutant mice and rat model. We have shown previously that *tnnt2a*^{-/-} mutated zebrafish exhibited phenotypes such as cardiac dilatation and asystole, which were similar to the clinical symptoms of patients with clinical DCM^[11]. Our results showed that *tnnt2a*^{-/-} zebrafish had distinct DCM phenotypes, such as cardiac enlargement and venous congestion, compared with normal controls. Since there are currently no ejection fraction assay instruments and techniques for zebrafish larvae, we confirmed the presence of ventricular dilation in this model zebrafish by measuring the sinus venosus-bulbus arteriosus (SV-BA) distance. Specifically, part of the zebrafish in the model1 and model2 groups died at 5dpf, and then the survival rate decreased significantly, the heart rate in the model 2 group decreased, and the SV-BA of the zebrafish in both model groups increased. The zebrafish in the model group became wider as a whole, with tissue edema, disordered arrangement, relaxation of muscle fibers, enlarged intercellular spaces, and an enlarged heart. These data demonstrate that the DCM zebrafish model is successfully replicated and can serve as a reproducible, replaceable, simple, and novel experimental animal model for DCM.

As we know, dilated cardiomyopathy is caused by a genetic defect, and there is currently no specific drugs for the treatment of the disease. This study is the first to explore drugs for the treatment of *tnnt2a* mutant DCM. In addition, we also used this model to study the effective traditional Chinese medicine compound Kuoxinfang and its sovereign drug *Astragalus mongholicus* Bunge (Huangqi), and found that it has a certain effect on the treatment of cardiomyopathy. Astragalus polysaccharide (APS) is an important active ingredient extracted from Huangqi, and has been widely used in the treatment of cardiovascular diseases^[31-32]. It has been reported that APS attenuates Iso-induced cardiac hypertrophy through regulating energy biosynthesis^[33].

In this study, we used *tnnt2a*^{-/-} mutated zebrafish to demonstrate that the cardioprotective effects of APS may be associated with the reduction of cardiomyocyte apoptosis. We determined the maximum tolerated concentration (MTC) of APS in zebrafish prior to the intervention study of APS. As a result, our data showed that zebrafish can tolerate it at a concentration of 15 µg/ml.

DCM is one of the leading causes of heart failure, and survival is poor^[34]. Therefore, relieving patients' symptoms and increasing survival time are the overall goals of the treatment of DCM. Our findings show that APS has certain efficacy in reducing mortality and improving symptoms when treating DCM zebrafish, which to a certain extent provides new ideas for clinical treatment. In addition, APS can significantly reduce the enlargement of DCM zebrafish heart and venous congestion area and can increase ATP content, cardiac bleeding volume, blood flow velocity, and improve heart rate. Our work provides clues for the treatment of DCM, but more research is needed for further confirmation.

Apoptosis is one of the ways leading to cardiomyocyte injury^[35]. The reduction of cardiomyocyte number and insufficient compensation of residual cardiomyocyte systolic function caused by apoptosis are important factors promoting the deterioration of left ventricular dysfunction and ventricular remodeling in DCM heart failure^[36]. Bax and Bcl-2 are endogenous mitochondrial apoptosis-related proteins^[37]. Caspase family mediates the apoptosis process mainly through mitochondrial pathway and membrane death receptor pathway, and is another key factor in the signal transduction of apoptosis process^[38]. Caspase-9, as the upstream initiating factor of Bcl-2 family, can activate the apoptosis executive factor Caspase-3, induce the disappearance of mitochondrial membrane potential, change the mitochondrial permeability, cause nucleic acid breakage, and finally lead to apoptosis^[39]. Throughout this study, TUNEL staining showed that the myocardial injury of zebrafish in the model group was closely related to apoptosis, and astragalus polysaccharide could reduce the apoptosis of zebrafish.

Conclusion

Our findings suggest that APS has a positive effect on cardio protection in the *tnnt2* mutant zebrafish model of DCM, which is dependent on the inhibition of cell apoptosis, this may provide a new idea for the treatment of dilated cardiomyopathy with gene mutation.

Abbreviations

DCM Dilated Cardiomyopathy

APS Astragalus polysaccharide

DOX Doxycycline

DEG Differential Expressed Genes

SV-BA sinus venosus-bulbus arteriosus

GO Gene Ontology

KEGG Kyoto Encyclopedia of Genes and Genomes

Declarations

Competing interests: The authors declare no competing interests.

Animal experiments were approved by the Fudan University Shanghai Medical School Animal Care and Use Committee.

Data Availability Statement

The original data supporting the conclusion of this article will be provided by the authors without reservation.

Author Contributions

CZ,HZ, XW, and YhW conceived and designed the experiments. CZ, HZ, LpP, and YdD contributed to performing the experiments. CZ,QW, YW, MjM, and MIZ analyzed the data. CZ, YhW and YjX wrote the manuscript. XW, YjX and YhW critically revised the manuscript.

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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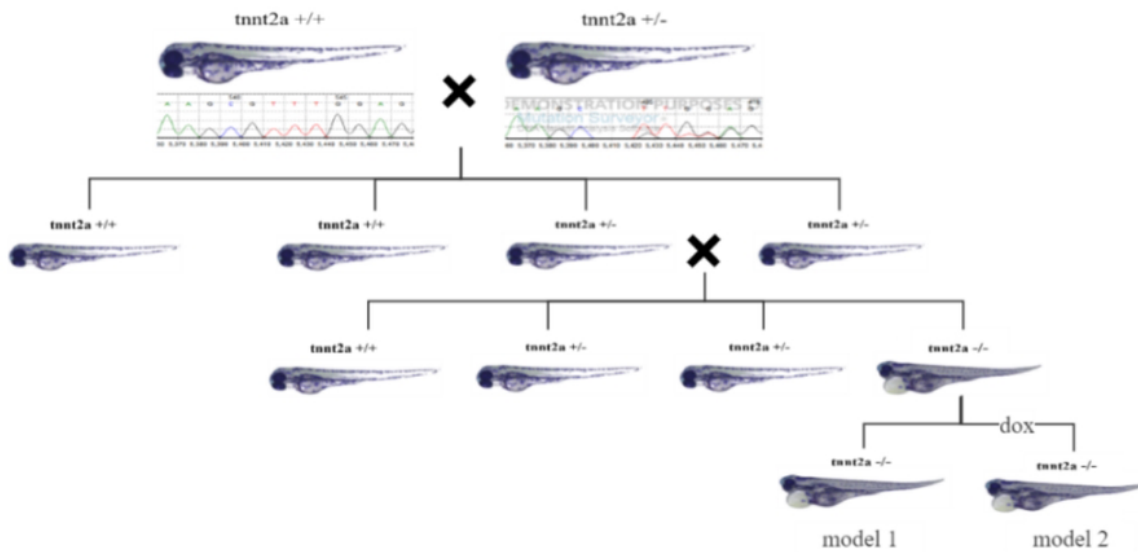
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Figures



B.

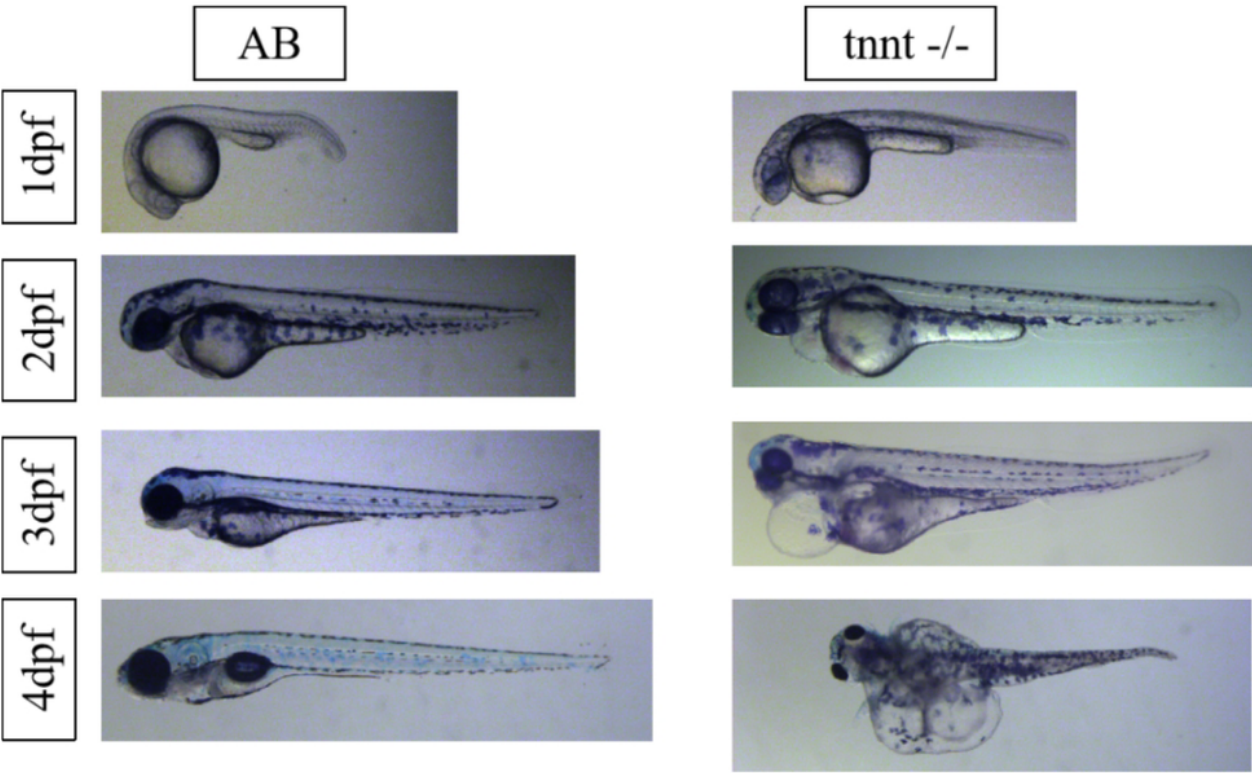


Figure 1

Acquisition and phenotypic analysis of $tnnt2a^{-/-}$ zebrafish.

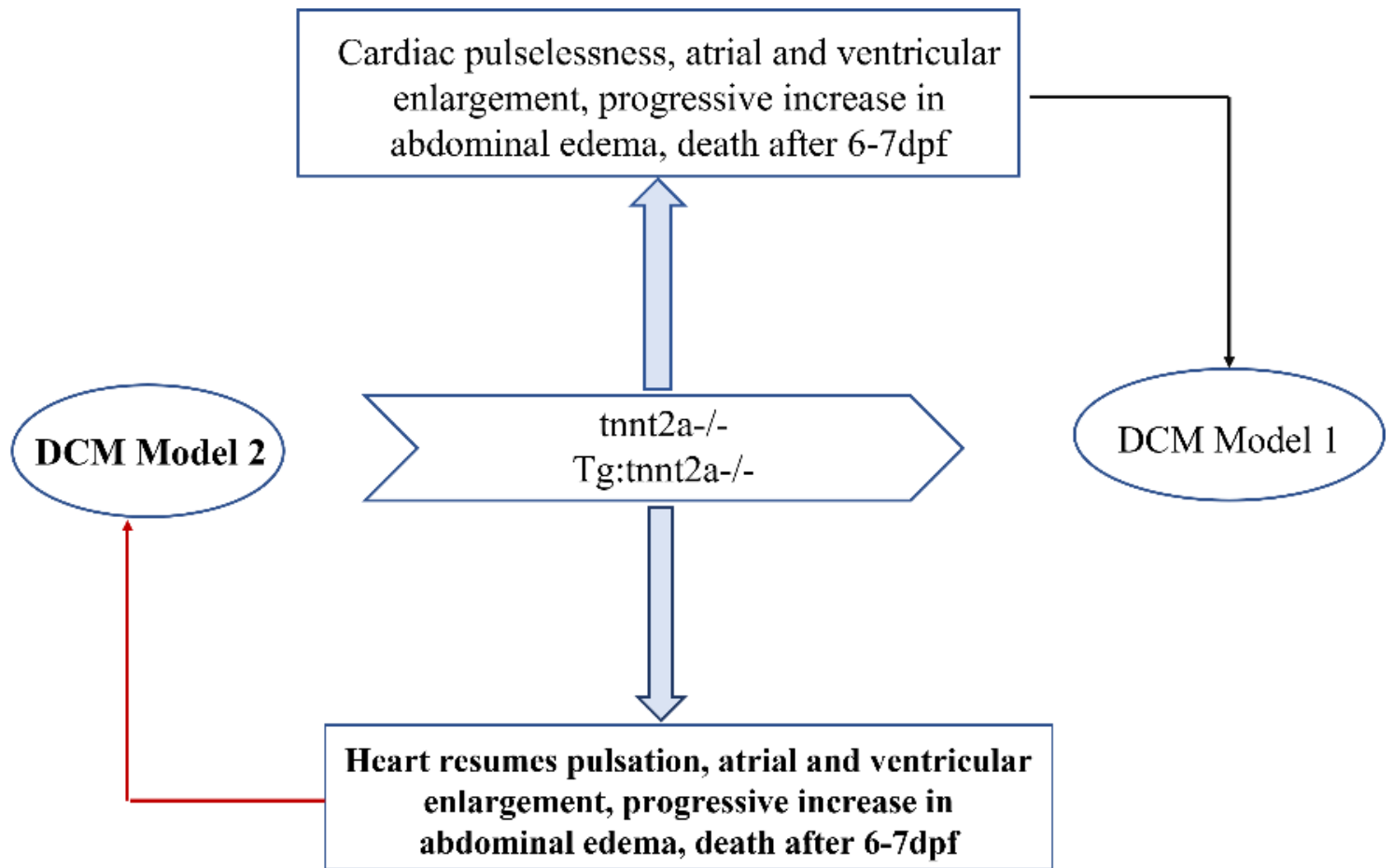


Figure 2

Phenotypic differences between model 1 and model 2

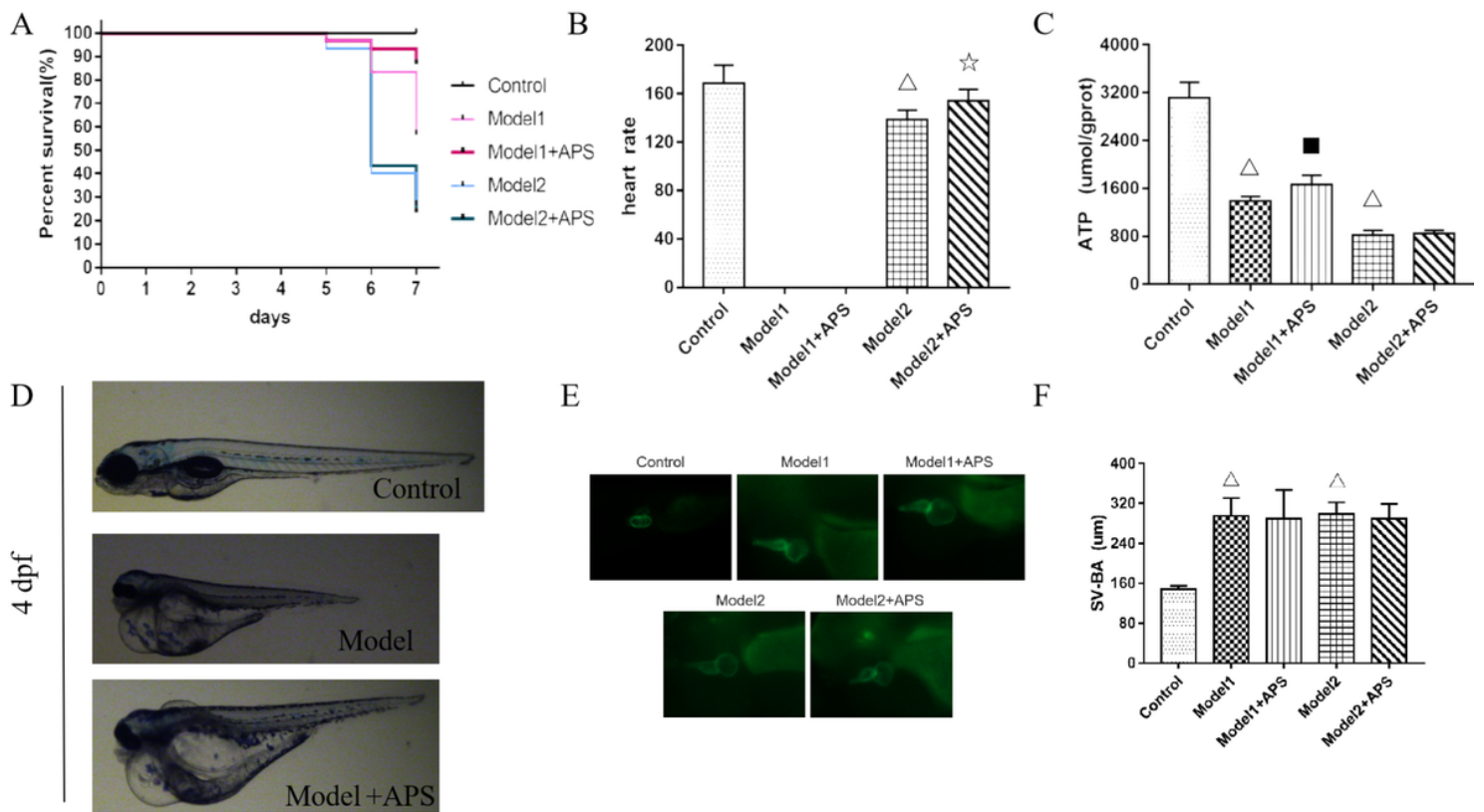


Figure 3

APS improves the cardiac function of DCM zebrafish.(A).The survival rates of zebrafish in each group. (B).The heart rate of zebrafish in model groups and after APS treatment. (C).Effects of APS on the ATP of zebrafish in model groups. (D).Representative images of zebrafish in each group at 4dpf. (E-F).Comparison of SV-BA of zebrafish in model groups and after APS treatment.Mean $\pm$ SD,n=30.The experiment was repeated three times. Δ P<0.01,compared with control, $\blacksquare$ P<0.05,compared with model 1, P<0.01, compared with the model2.

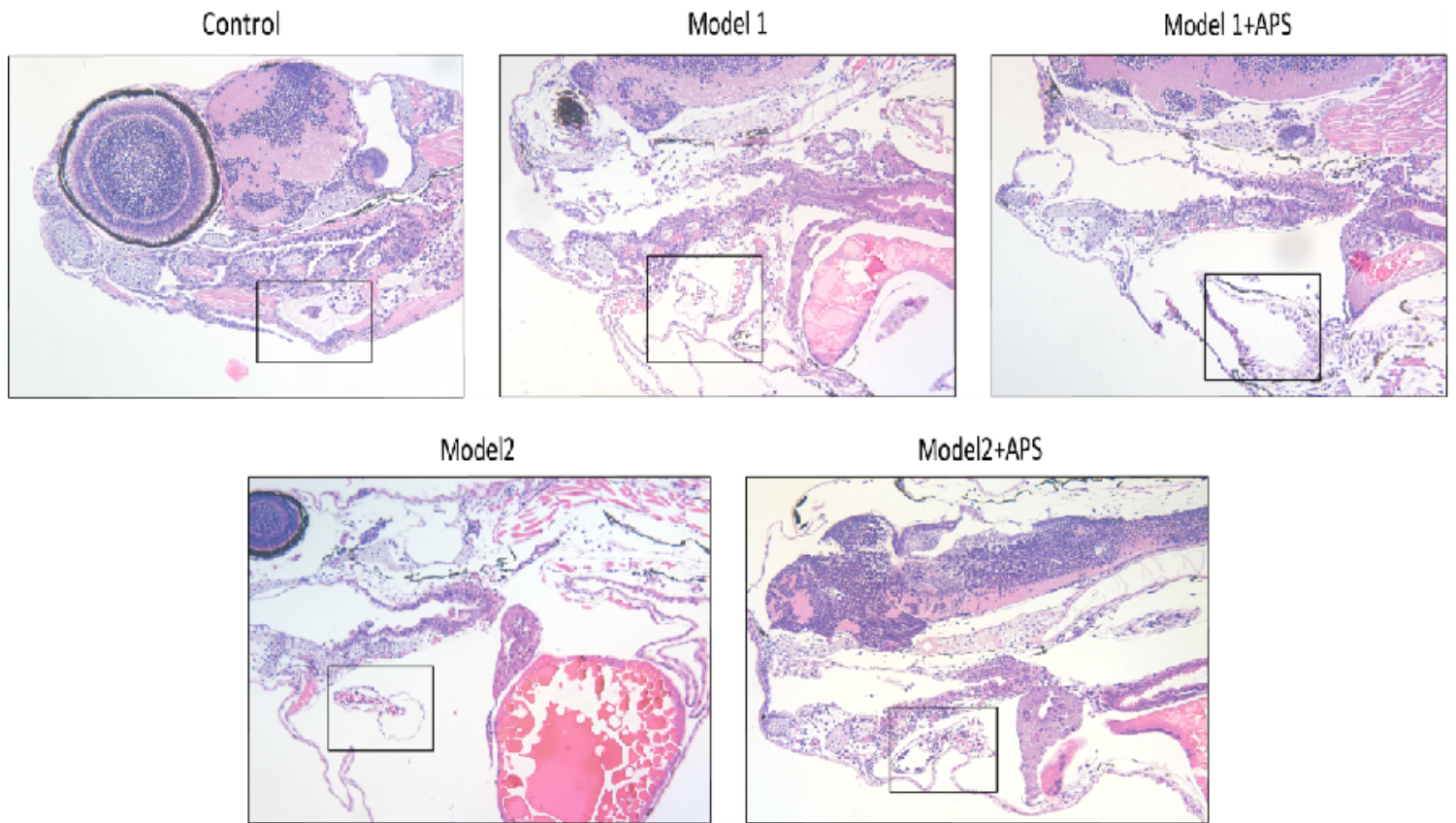


Figure 4

APS reduces swelling to protect myocardial cells from injury on DCM Zebrafish. zebrafish was taken for HE staining analysis.

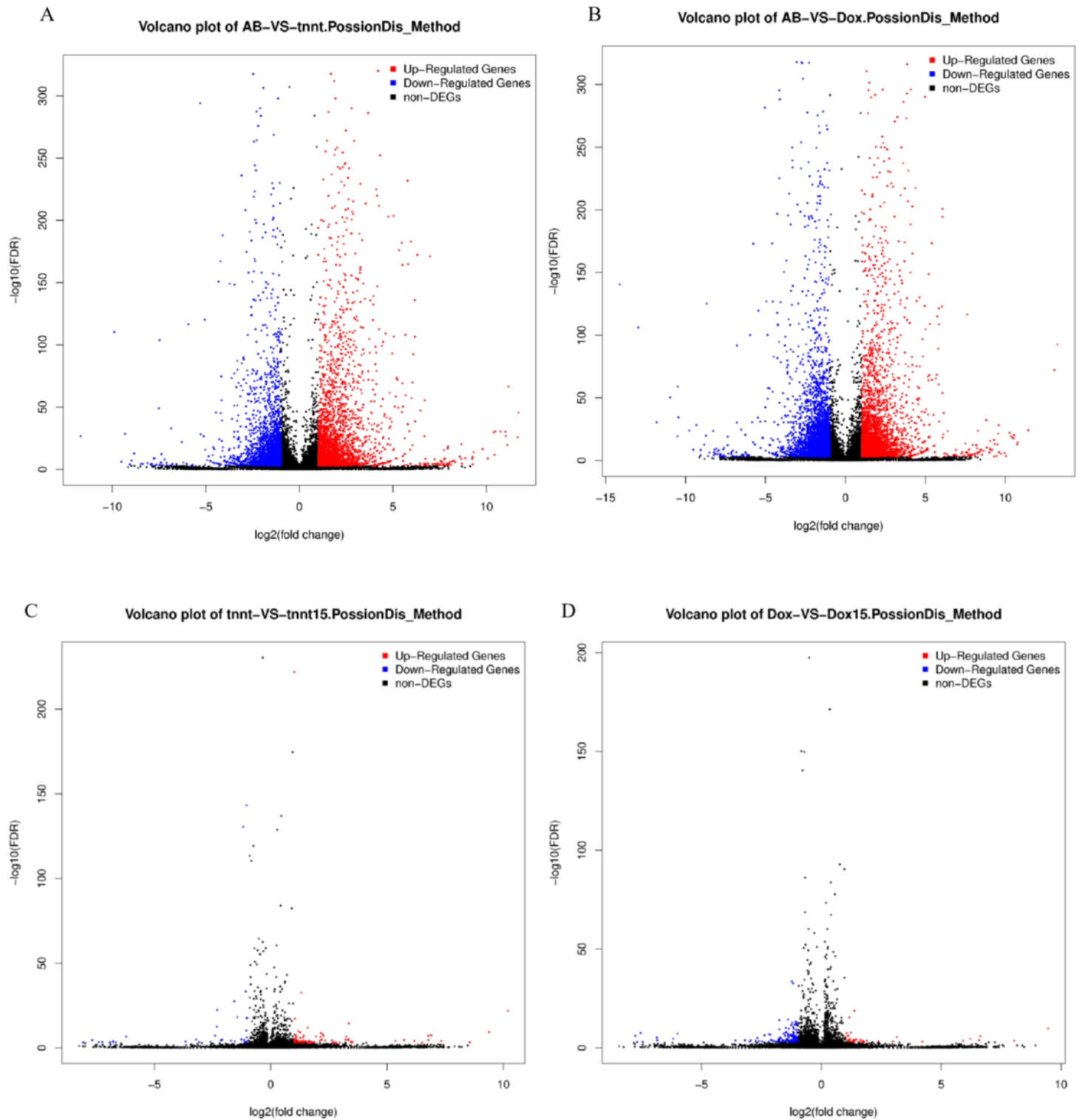


Figure 5

After 48 hours of APS intervention, Danio rerio were harvested and subjected to RNA-seq analysis. (A).Differentially expressed genes of AB vs.DOX(B).Differentially expressed genes of AB vs.Tntt. (C).Differentially expressed genes of DOX vs.DOX15(D).Differentially expressed genes of tntt vs. Tntt15. $|\log_2(\text{FoldChange})| > 1, p \text{ value} < 0.001$.

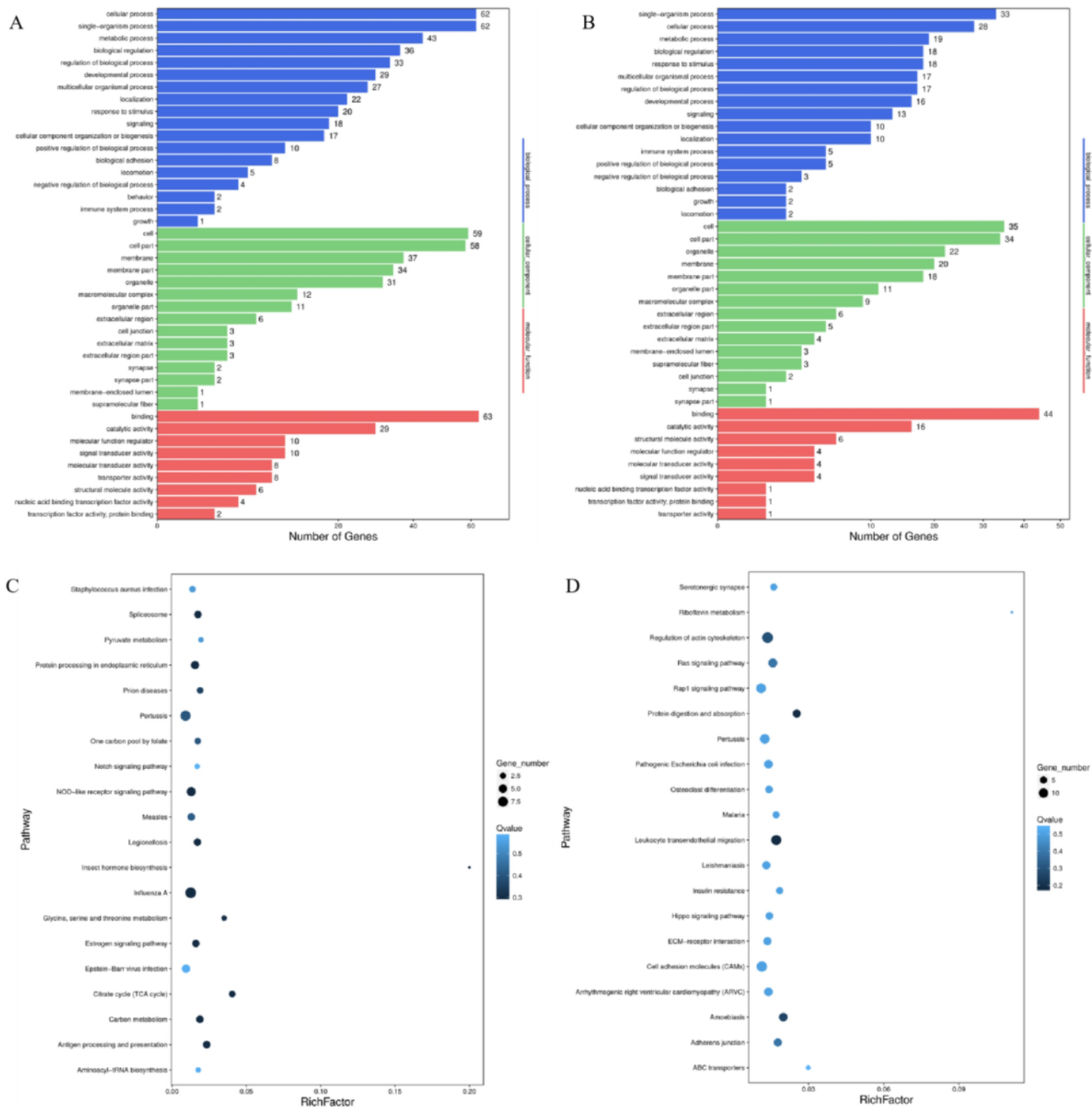


Figure 6

(A,B).GO analysis of the top 30 functions impacted by *tnnt15* and *DOX15*.(C,D)The KEGG analysis of top 20 pathways of the genes impacted by *tnnt15* and *DOX15*. The pathway functions were ranked from left to right by $-\log(P\text{-value adjusted})$.

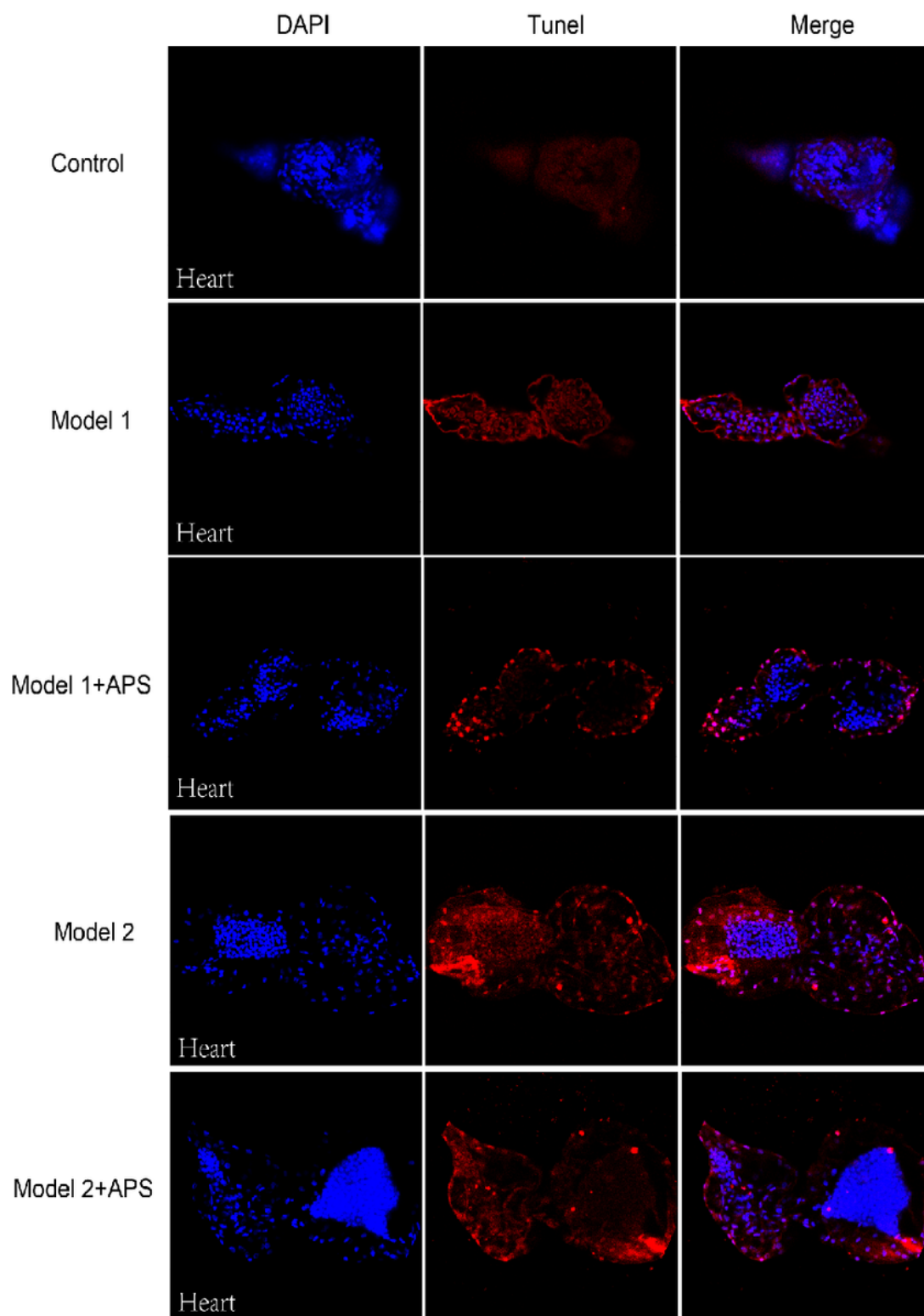


Figure 7

Effects of APS on the cardiomyocytes apoptosis of zebrafish in model groups.TUNEL staining of zebrafish hearts showing the apoptotic cells (red indicates apoptotic cells and blue indicates nuclei).

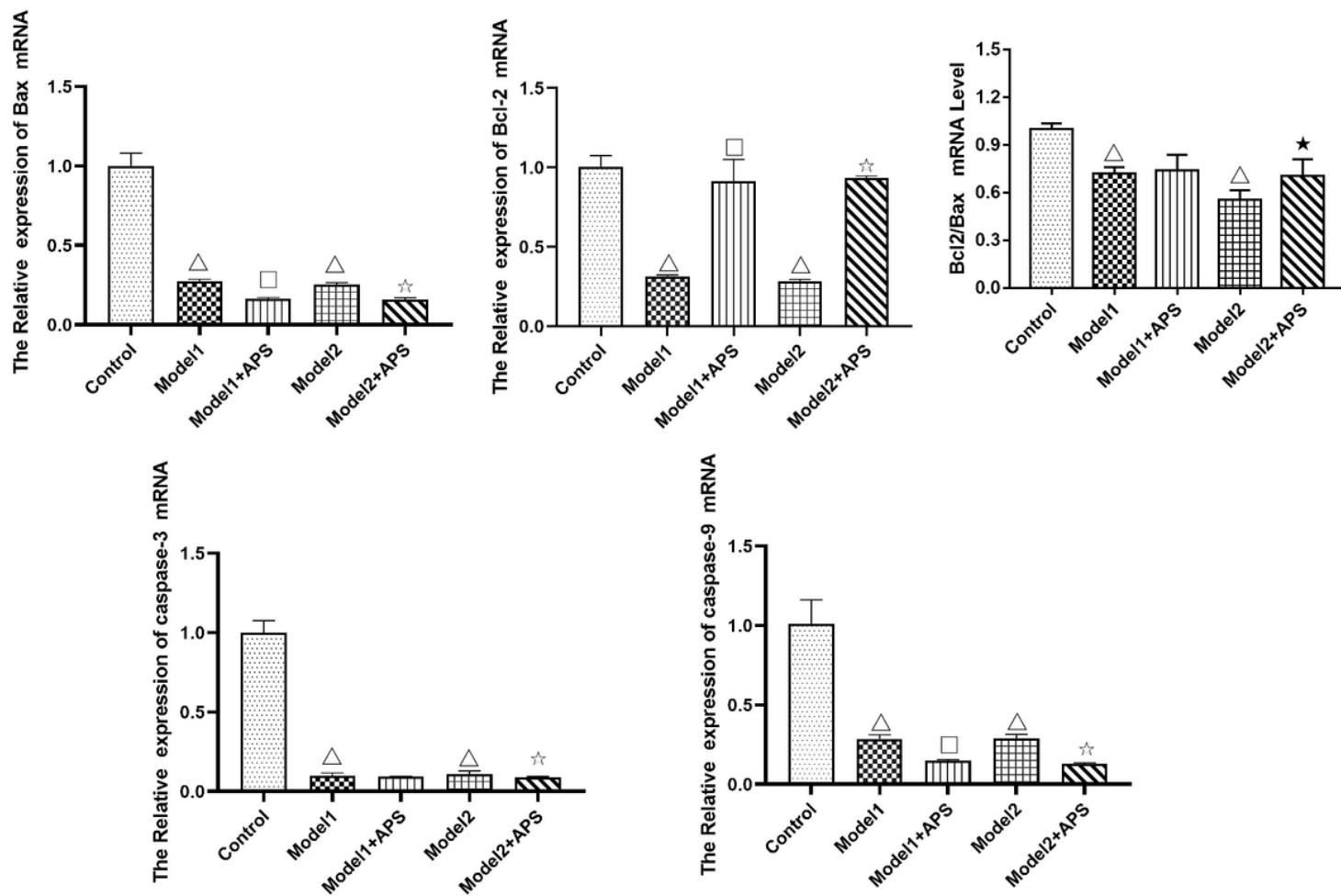


Figure 8

Comparison of mRNA expression of Bax,Bcl-2,Bcl2/Bax,caspase-3 and caspase-9 of DCM zebrafish treated with APS.ΔP 0.01,Compared with control; P<0.01,compared with model 1; P 0.01,compared with model 2; P 0.05,compared with model 2.