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mmu-miR-146a-5p and mmu-miR-122-5 via targeting the multiple target genes played an important role in the Huangqi treatment of diabetes mellitus

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Abstract

Background To explore the molecular mechanism of exercise and Huangqi on the effects of diabetes mellitus were examined for miRNA sequencing.

Mehtods Mouses in the model control group were fed with normal saline, mouses in the Huangqi group were intragastrically fed with huangqi for every day for 6 weeks, mouses in the Swimming group were trained with swimming per three days for for 6 weeks, and mouses in the Combine treatment groups were intragastrically fed with huangqi for every day for 6 weeks and trained with swimming per three days. We profiled the miRNA expression of skeletal muscle and myocardial tissues of mouse in those groups. Differentially expressed miRNAs were screened, and the databases, such as miRanda, miRDB, Targetscan and miRTarBase, were used to predict the target genes of differentially expressed miRNAs. Function alanalysis of the target genes of differentially expressed miRNAs was performed using Gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) database.

Results In this study, mmu-miR-193b-3p, mmu-miR-5115, and mmu-miR-146a-5p were significantly differential expression in among groups in the skeletal muscle tissue. In the myocardial tissue, those miRNAs, such as mmu-miR-122-5p, mmu-miR-146a-5p and mmu-miR-215-5p were significantly differential expression in among groups. The miRNA-target genes interaction network was constructed to further investigate interaction correlations.

Conclusion The results implied that mmu-miR-146a-5p and mmu-miR-122-5 played an important role in the treatment of diabetes mellitus, via targeting the multiple target genes.

Keywords: diabetes mellitus; miRNA sequencing; exercise, Huangqi

Background

Obesity is strongly correlated with type 2 diabetes mellitus, a common disorder of glucose and lipid metabolism [1]. Sporting activity was becoming a common practice in patients with diabetes mellitus (DM), and exercise was widely perceived to be beneficial for glycemic control and weight loss in patients with type 2 diabetes [2, 3]. Astragalus membranaceus is one of the most important traditional Chinese medicinal herbs because it contains triterpenoid saponins, which have beneficial and pharmacological effects on health [4]. Some researchers reported that Huangqi Xiaoke formula was effective on relieving traditional Chinese medical symptoms in T2DM patients with deficiency of both qi and yin, especially on relieving symptoms of dry throat, lassitude, shortness of breath and unwilling to speak [5]. However, exercise (such as swimming) and Huangqi on the effects of diabetes mellitus have few reported.

miRNAs, with an average length between 18 and 26 nt [6], function as negative regulators of gene expression by inhibiting translation or regulating mRNA degradation [7]. miRNAs act by binding to the 3'-untranslated regions (3'-UTR) of target gene transcription products [8] but can also induce gene expression by targeting promoter sequences [9]. Although miRNAs are encoded by only a small number of genes, they may regulate the expression

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of many mRNAs [10, 11]. Increasing evidence has suggested that miRNAs are involved in cell differentiation [12], cell proliferation [13], and cell apoptosis [14].

The differences in miRNA profiles for the exercise (such as swimming) and Huangqi on the effects of diabetes mellitus have not been reported. Therefore, in this study, we used miRNA sequencing to explore the regulation of exercise and Huangqi on the effects of diabetes mellitus. Systematic analyses were carried out of the differentially expressed miRNAs, as well as their target genes, to provide insights into the mechanisms of miRNA regulation in the treatment for diabetes mellitus.

Methods

Animals

A total of 28 male or db/db mouse (weight 12-15 g) provided by Model Animal Research Center of Nanjing University (Nanjing, Jiangsu, China) were randomly divided into 5 groups after being fed adaptively with normal diets. Mouse in the model control group were fed with normal saline (0.9% NS, 20ml/kg, n = 7), mouse in the Huangqi group were intragastrically fed with huangqi for every day for 6 weeks (5ml/kg, n = 7), mouse in the Swimming group were trained with swimming per three days for for 6 weeks (n = 7), and mouse in the Combine treatment groups were intragastrically fed with huangqi for every day for 6 weeks and trained with swimming per three days (n = 7). Then, the mouse was executed, and skeletal muscle and myocardial tissues were preserved in liquid nitrogen and transferred to - 80 ℃ refrigerator for preservation.

Library construction and sequencing

Total RNA was isolated and purified using Trizol reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's procedure. The RNA amount and purity were quantified using NanoDrop ND-1000 (NanoDrop, Wilmington, DE, USA). The RNA integrity was assessed by Agilent 2100, and the RNA with RIN >7.0 was used for the subsequent sequencing. The RNAs were fragmented into small pieces, and then the cleaved RNA fragments were reverse-transcribed to create the cDNA. At last, we performed the paired-end sequencing on an Illumina Hiseq 4000 following the vendor's recommended protocol.

Data preprocessing

Cutadapt [15] was used to remove the reads that contained adaptor contamination, low quality bases and undetermined bases. Then sequence quality was verified using FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). We used Mirdeep2 to map reads to the genome of mmu10, estimate the expression levels of miRNAs and predict to the novel miRNAs [16].

Different expression analysis of miRNAs and target gene prediction

edgeR was used to calculate the expression level for miRNAs [17]. The differentially expressed miRNAs were selected with log2 (fold change) > 1 or log2 (fold change) < -1 and with statistical significance (p value < 0.05). The databases, such as miRanda, miRDB, Targetscan and miRTarBase, were used to predict the target genes of differentially expressed miRNAs.

Functional analysis

Function alanalysis of the target genes of differentially expressed miRNAs was performed using GO annotation (http://www.geneontology.org/), and the genes were categorized according to their biological process, molecular function and cellular component [18]. The target genes of differentially expressed miRNAs were further assigned to KEGG database (http://www. genome.jp/kegg/pathway.html). Significantly altered GO terms and KEGG pathways were identified based on a hypergeometric test.

Results

Analysis of differentially expressed miRNAs

In the skeletal muscle tissue, the expression of 4 miRNAs changed (2 increased, 2 decreased; $P < 0.05$) between swimming group and model control group, whereas 72 changed (38 increased, 34 decreased; $P < 0.05$) between huangqi group and model control group, and 4 changed (3 increased, 1 decreased; $P < 0.05$) between combine treatment group and model control group (Figure 1 and Additional table 1). Those miRNAs, such as mmu-miR-193b-3p, mmu-miR-5115, and mmu-miR-146a-5p were significantly differential expression in among groups.

In the myocardial tissue, the expression of 5 miRNAs changed (3 increased, 2 decreased; $P < 0.05$) between swimming group and model control group, whereas 3 changed (2 increased, 1 decreased; $P < 0.05$) between huangqi group and model control group, and 5 changed (3 increased, 2 decreased; $P < 0.05$) between combine treatment group and model control group (Figure 2 and Additional table 2). Those miRNAs, such as mmu-miR-122-5p, mmu-miR-146a-5p and mmu-miR-215-5p were significantly differential expression in among groups.

Target gene prediction and functional analysis of different expression miRNAs

The differentially expressed miRNAs were selected, and the databases, such as miRanda, miRDB, Targetscan and miRTarBase, were used to predict the target genes of differentially expressed miRNAs. Function alanalysis of the target genes of differentially expressed miRNAs was performed using GO and KEGG database.

In the skeletal muscle tissue, the target genes of differentially expressed miRNAs in swimming group and model control group were enriched in antigen processing and presentation of peptide antigen via MHC class I, antigen processing and presentation of peptide antigen, antigen processing and presentation, antigen processing and presentation of endogenous peptide antigen via MHC class I, antigen processing and presentation of endogenous peptide antigen, antigen processing and presentation of endogenous antigen, positive regulation of T cell mediated cytotoxicity, regulation of T cell mediated cytotoxicity, positive regulation of T cell mediated immunity, and T cell mediated cytotoxicity (Figure 3A). Pathways, such as Allograft rejection, Graft-versus-host disease, Type I diabetes mellitus, Autoimmune thyroid disease, Viral myocarditis, Antigen processing and presentation, Cell adhesion molecules (CAMs), Phagosome, Cellular senescence, Kaposi's sarcoma-associated herpesvirus infection, Herpes simplex infection, Epstein-Barr virus infection, Viral carcinogenesis, HTLV-I infection, Endocytosis, Human papillomavirus infection, Complement and coagulation cascades and Natural killer cell mediated cytotoxicity were enriched (Figure 3B). The target genes of differentially expressed miRNAs in huangqi group and model control group were enriched in antigen processing and presentation of peptide antigen via MHC class I, antigen processing and presentation of peptide antigen, antigen processing and presentation, blood coagulation, hemostasis, coagulation, antigen processing and presentation of endogenous peptide antigen, wound healing, antigen processing and presentation of endogenous antigen and fibrinolysis (Figure 3C). Pathways, such as Allograft rejection, Graft-versus-host disease, Type I diabetes mellitus, Autoimmune thyroid disease, Viral myocarditis, Antigen processing and presentation, Epstein-Barr virus infection, Cell adhesion molecules (CAMs), Phagosome, Cellular senescence, Viral carcinogenesis, Kaposi's sarcoma-associated herpesvirus infection, Herpes simplex infection, HTLV-I infection, Endocytosis, Human papillomavirus infection, Complement and coagulation cascades, RNA polymerase and Cytosolic DNA-sensing pathway were enriched (Figure 3D). The target genes of differentially expressed miRNAs in combine treatment group and model control group were enriched in antigen processing and presentation of peptide antigen via MHC class I, antigen processing and presentation of peptide antigen, antigen processing and presentation, wound healing, antigen processing and presentation of endogenous peptide antigen via MHC class I, proteasome assembly, response to wounding, antigen processing and presentation of endogenous peptide antigen, positive regulation of histone deacetylation and antigen processing and presentation of endogenous antigen (Figure 3E). Pathways, such as Epstein-Barr virus infection, Allograft rejection, Viral carcinogenesis, Graft-versus-host disease, Type I diabetes mellitus, Autoimmune thyroid disease, Viral myocarditis, Antigen processing and presentation, Cell adhesion molecules (CAMs), Phagosome, Cellular senescence, Kaposi's sarcoma-associated herpesvirus infection, Herpes simplex infection, HTLV-I infection, Endocytosis, Human papillomavirus infection, Complement and coagulation cascades and Basal transcription factors were enriched (Figure 3F).

In the myocardial tissue, the target genes of differentially expressed miRNAs in swimming group and model control group were enriched in antigen processing and presentation of peptide antigen via MHC class I, antigen processing and presentation of peptide antigen, antigen processing and presentation, blood coagulation, hemostasis, coagulation, antigen processing and presentation of endogenous peptide antigen, wound healing, antigen processing and presentation of endogenous antigen and fibrinolysis (Figure 4A). Pathways, such as Allograft rejection, Graft-versus-host disease, Type I diabetes mellitus, Autoimmune thyroid disease, Viral myocarditis, Antigen processing and presentation, Epstein-Barr virus infection, Viral carcinogenesis, Cell adhesion molecules (CAMs), Phagosome, Cellular senescence, Kaposis sarcoma-associated herpesvirus infection, Herpes simplex infection, HTLV-I infection, Endocytosis, Human papillomavirus infection and Natural killer cell mediated cytotoxicity were enriched (Figure 4B). The target genes of differentially expressed miRNAs in huangqi group and model control group were enriched in antigen processing and presentation of peptide antigen via MHC class I, antigen processing and presentation of peptide antigen, antigen processing and presentation, antigen processing and presentation of endogenous peptide antigen

via MHC class I, antigen processing and presentation of endogenous peptide antigen, antigen processing and presentation of endogenous antigen, positive regulation of T cell mediated cytotoxicity, regulation of T cell mediated cytotoxicity, positive regulation of T cell mediated immunity, and T cell mediated cytotoxicity (Figure 4C). Pathways, such as Allograft rejection, Graft-versus-host disease, Type I diabetes mellitus, Autoimmune thyroid disease, Viral myocarditis, Antigen processing and presentation, Epstein-Barr virus infection, Viral carcinogenesis, Cell adhesion molecules (CAMs), Phagosome, Cellular senescence, Kaposis sarcoma-associated herpesvirus infection, Herpes simplex infection, HTLV-I infection, Endocytosis, Human papillomavirus infection, Complement and coagulation cascades and Natural killer cell mediated cytotoxicity were enriched (Figure 4D). The target genes of differentially expressed miRNAs in combine treatment group and model control group were enriched in antigen processing and presentation of peptide antigen via MHC class I, antigen processing and presentation of peptide antigen, antigen processing and presentation, antigen processing and presentation of endogenous peptide antigen via MHC class I, blood coagulation, hemostasis, coagulation, antigen processing and presentation of endogenous peptide antigen, antigen processing and presentation of endogenous antigen and positive regulation of T cell mediated cytotoxicity (Figure 4E). Pathways, such as Allograft rejection, Graft-versus-host disease, Type I diabetes mellitus, Autoimmune thyroid disease, Viral myocarditis, Antigen processing and presentation, Epstein-Barr virus infection, Viral carcinogenesis, Cell adhesion molecules (CAMs), Phagosome, Cellular senescence, Kaposis sarcoma-associated herpesvirus infection, Herpes simplex infection, HTLV-I infection, Endocytosis, Human papillomavirus infection, Complement and coagulation cascades and Natural killer cell mediated cytotoxicity were enriched (Figure 4F).

Interaction analysis

Those miRNAs, such as mmu-miR-193b-3p, mmu-miR-5115, mmu-miR-146a-5p, mmu-miR-122-5p and mmu-miR-215-5p were significantly differential expression in among groups. To further focus on the role of miRNAs, the miRNA-target genes interaction network was constructed to further investigate interaction correlations. The target genes of mmu-miR-146a-5p were IRAK1, Camk2a, Ifng, Cd93, Map1b, Nos2, Notch1, Nrp2, Med1, Relb, Stat1, Tnni1, Traf6, RNF11, Rsad2, Slc47a1, Mllt3, Ndor1, Irak2, Sgk3 and Gpr157. The target genes of mmu-miR-122-5 were Chrna1, Afp, Alpl, Alas2, Aldoa, Slc7a1, B2m, Bach1, Bckdk, Bmpr1a, Camk2b, Ccnd1, Ccng1, Ccrn4l, Cpox, Cs, Csf3r, Cyp2b13, Dbp, Ddc, E2f1, Fech, Ctgf, Ggps1, Gys1, H2-T24, Hba-a1, Hfe, Hmbs, Igf2, Il1b, Jun, Smad4, Smad7, P4ha1, Per1, Pkm, Ppox, Prom1, Rbl2, Ccl2, Sox4, Tgfbr1, Tfrc, Urod, Uros, Klf6, Ndrg3, Hist1h1c, Tfr2, Irf6, Cd320, Rcan1, Paxip1, Cxcl13, Gde1, Zfp113, Xpo7, Tmed3, Tmem206, Gpx7, Slc35a4, Sgol2, 5-Mar, Hfe2, Zfp949, Nanog, Thap1, Tmem50b, Smarcd1, Hamp, Sfxn4, Rell1, Sbk1, Sec23ip, Tfdp2, Iffo2, Cdh12, Socs2, Ppp1r16b, Apob, Slc35g1, Cers6, Snhg11, Mir451 and Mir17. In particular, mmu-miR-146a-5p and mmu-miR-122-5 played an important role in the development of diabetes mellitus (Figure 5).

Discussion

Obesity is strongly correlated with type 2 diabetes mellitus, a common disorder of glucose and lipid metabolism [1]. Sporting activity was becoming a common practice in patients with diabetes mellitus (DM), and exercise was widely perceived to be beneficial for glycemic control and weight loss in patients with type 2 diabetes [2, 3]. Some researchers reported that Huangqi Xiaoke formula was effective on relieving traditional Chinese medical symptoms in T2DM patients with deficiency of both qi and yin [5]. However, exercise (such as swimming) and Huangqi on the effects of diabetes mellitus have few reported. In this study, mmu-miR-193b-3p, mmu-miR-5115, and mmu-miR-146a-5p were significantly differential expression in among groups in the skeletal muscle tissue. In the myocardial tissue, those miRNAs, such as mmu-miR-122-5p, mmu-miR-146a-5p and mmu-miR-215-5p were significantly differential expression in among groups. To further focus on the role of miRNAs, the miRNA-target genes interaction network was constructed to further investigate interaction correlations. In particular, mmu-miR-146a-5p and mmu-miR-122-5 played an important role in the development of diabetes mellitus.

In the previously reports, some researchers have reported that mmu-miR-146a was up-regulated in the in adipose tissue after long-term high-fat diet-induced obesity in mice [19]. The miR-146a rs2910164 and miR-155 rs767649 polymorphisms were analyzed in 490 T1DM patients and in 469 nondiabetic subjects, and the results showed that it was associated with protection for T1DM, and the strongest association was observed for the dominant model [20]. miR-

146a rs2910164 polymorphism might be associated with carotid vulnerable plaque risk in Chinese type 2 diabetes mellitus patients, particularly in older patients, females, those with diabetes duration of more than 10 years and those with hypertension [21]. The presence of SNPs in in miR-27a, miR-146a,and miR-124a with T2DM type 2 diabetes mellitus (T2DM) among a Chinese population might promote disease by affecting miRNA expression and gene function [22-24]. MicroRNA-146a was considered as a comprehensive indicator of inflammation and oxidative stress status induced in the brain of chronic T2DM Rats [25]. In addition, a significant change was observed in the peripheral blood miRNA expression profile after Roux-en-Y gastric bypass (RYGB) surgery compared with those before operation, and the expression levels of hsa-miR-29a-3p, hsa-miR-122-5p, hsa-miR-124-3p, and hsa-miR-320a were downregulated in patients with type 2 diabetes mellitus (T2DM) [26]. Based on the above statements, the results showed that mmu-miR-146a-5p and mmu-miR-122-5 through targeting the multiple target genes played an important role in the treatment of diabetes mellitus.

Conclusion

In this study, mmu-miR-193b-3p, mmu-miR-5115, and mmu-miR-146a-5p were significantly differential expression in among groups in the skeletal muscle tissue. In the myocardial tissue, those miRNAs, such as mmu-miR-122-5p, mmu-miR-146a-5p and mmu-miR-215-5p were significantly differential expression in among groups. The miRNA-target genes interaction network was constructed to further investigate interaction correlations. The results implied that mmu-miR-146a-5p and mmu-miR-122-5 played an important role in the treatment of diabetes mellitus, via targeting the multiple target genes.

Abbreviations

Gene ontology (GO)

Kyoto Encyclopedia of Genes and Genomes (KEGG)

diabetes mellitus (DM),

3’-untranslated regions (3’-UTR)

Cell adhesion molecules (CAMs)

type 2 diabetes mellitus (T2DM)

Roux-en-Y gastric bypass (RYGB)

Ethics approval and consent to participate

Not applicable.

Consent for publication

Manuscript is approved by all authors for publication.

Availability of data and materials

The data and materials of this experiment are available.

Competing interests

No conflict of interest exits in this manuscript.

Fund：

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Authors' contributions

Xiujuan Liu and Nianyun Zhang were responsible for the design of the whole study. Nianyun Zhang and Bin Wang were in charge of experimental operation and drawing. Bin Wang was responsible for writing the manuscript. Biao Sun submitted the manuscripts.

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Not applicable.

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Figure legends

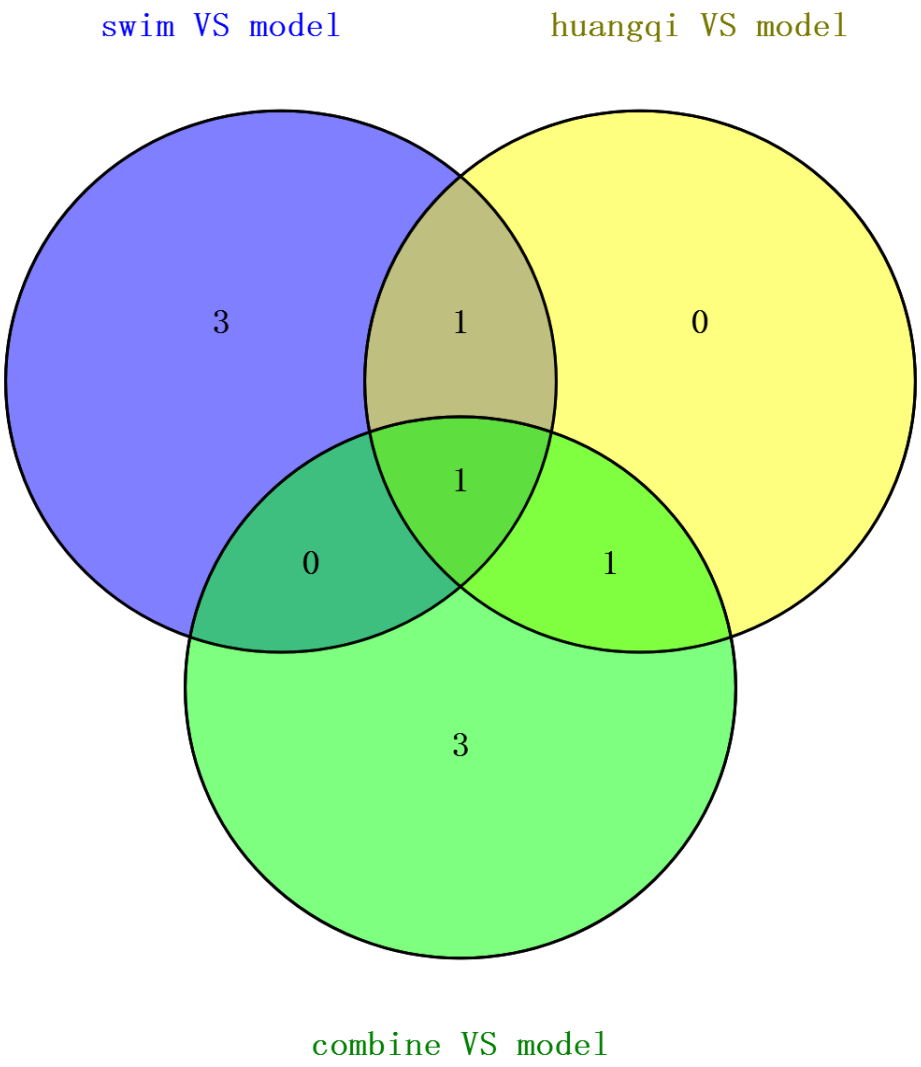

Figure 1. **Venn diagram illustrating of the differentially expressed miRNAs in the skeletal muscle tissue in the swimming group and model control group, huangqi group and model control group, and combine treatment group and model control group.**

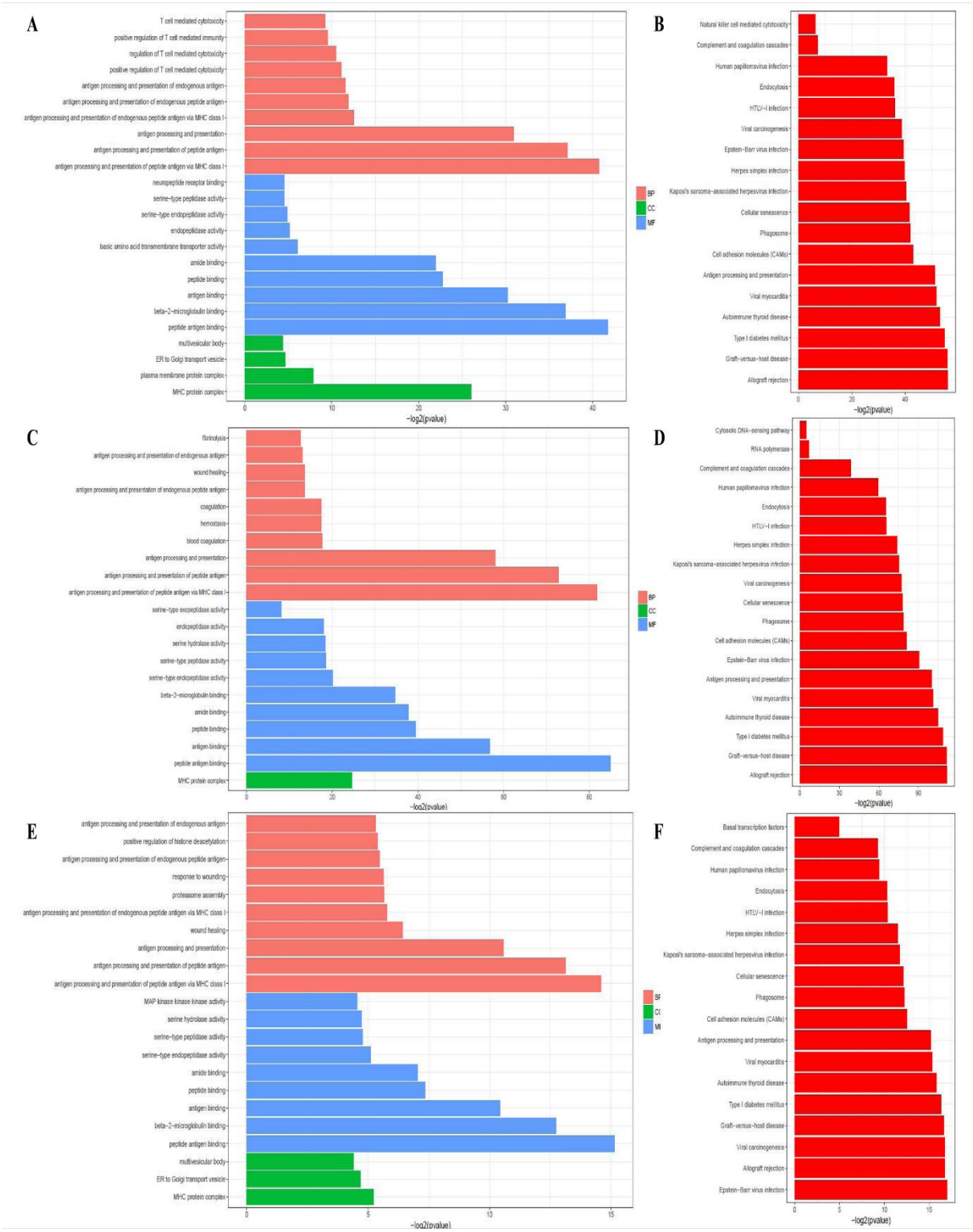

Figure 2. **Venn diagram illustrating of the differentially expressed miRNAs in themyocardial tissue in the swimming group and model control group, huangqi group and model control group, and combine treatment group and model control group.**

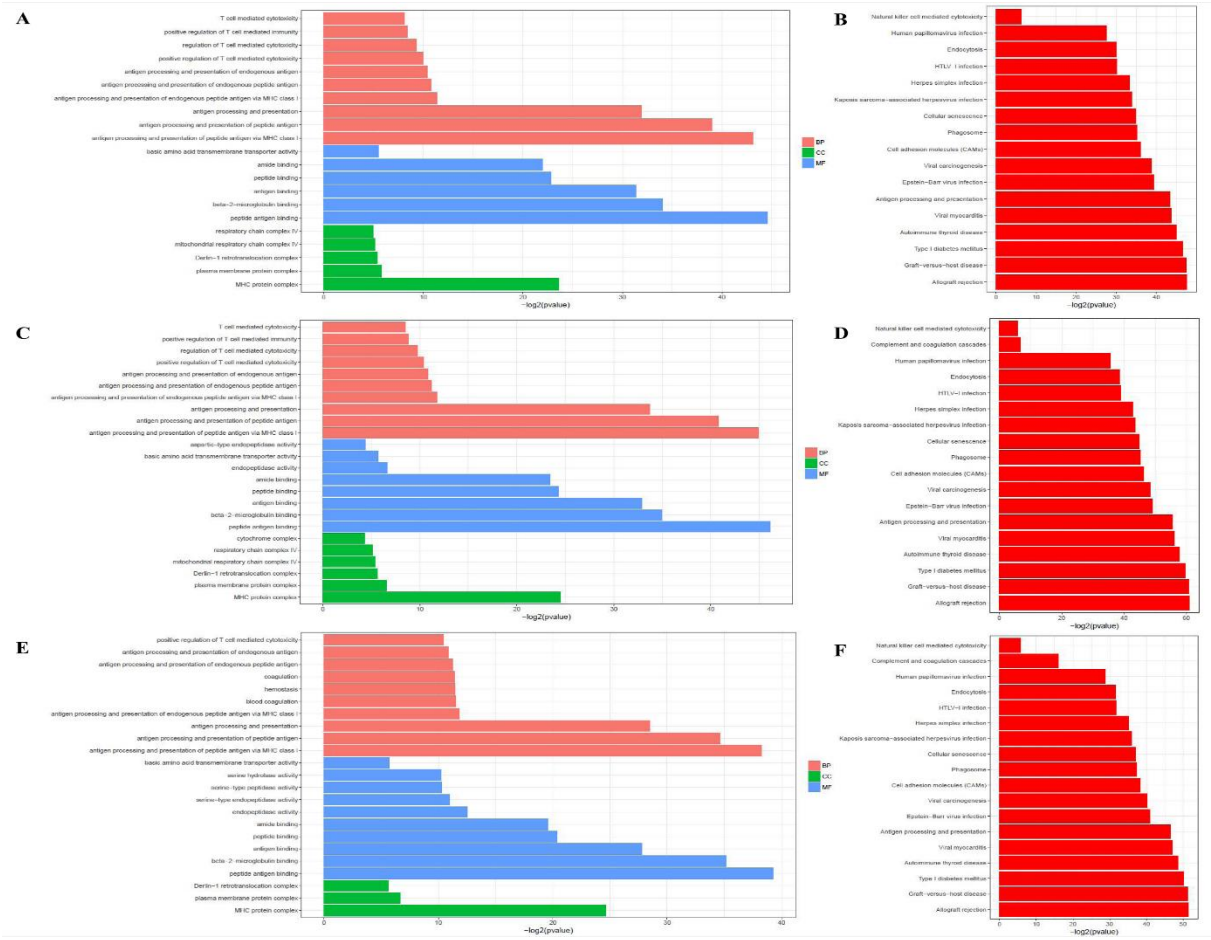

Figure 3. **GO enrichment analysis for the target genes of differentially expressed miRNAs in the skeletal muscle tissue in the swimming group and model control group (A), huangqi group and model control group (C), and combine treatment group and model control group (E). BP, biological process; CC, cellular component; MF, molecular function. Kyoto Encyclopedia of Genes and Genomes enriched pathway analysis of differentially expressed miRNAs in the skeletal muscle tissue in the swimming group and model control group (B), huangqi group and model control group (D), and combine treatment group and model control group (F). Enrichment analysis was performed by Fisher's exact test. *P* < 0.05 was used as the threshold.**

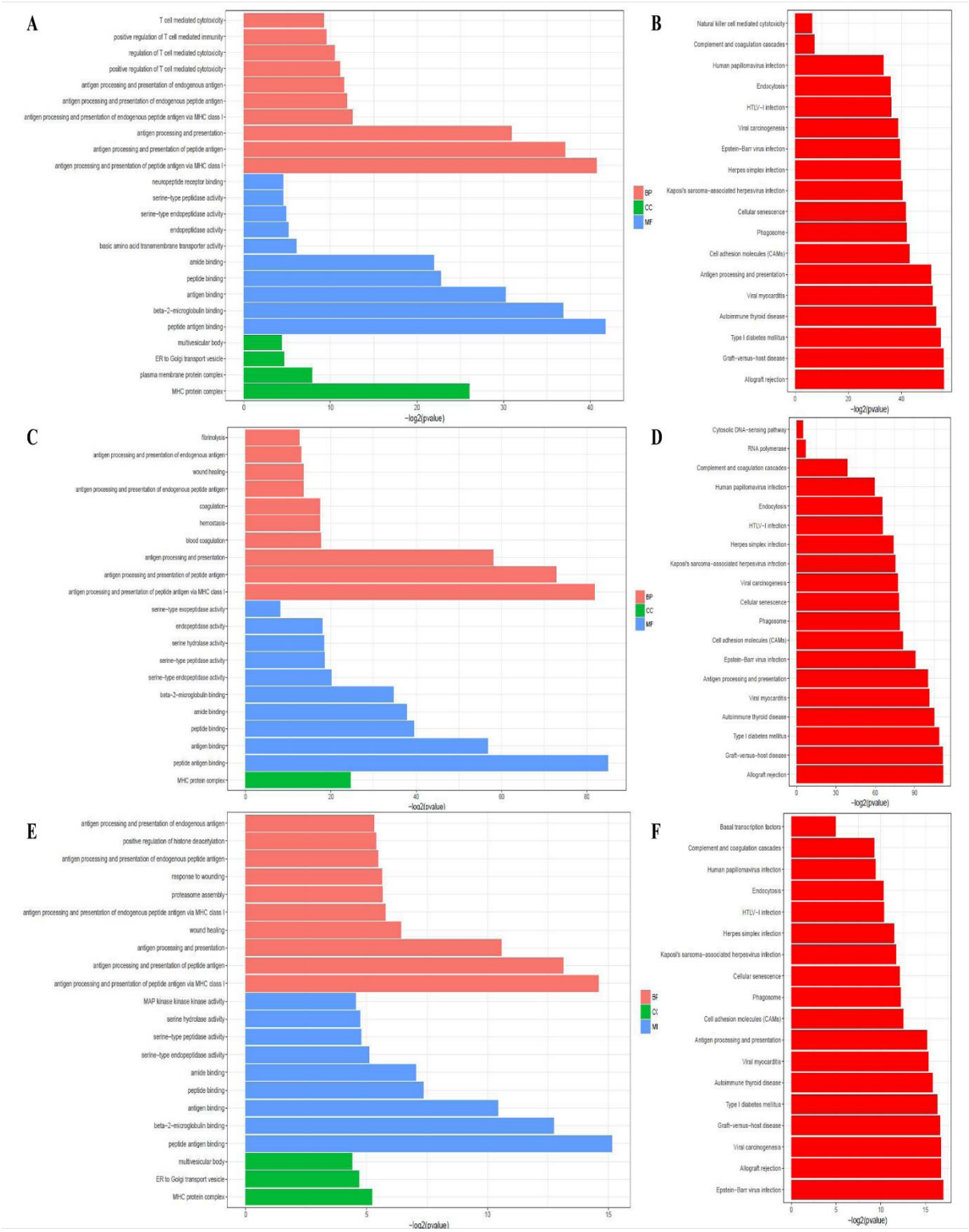

Figure 4. **GO enrichment analysis for the target genes of differentially expressed miRNAs in the myocardial tissue in the swimming group and model control group (A), huangqi group and model control group (C), and combine treatment group and model control group (E). BP, biological process; CC, cellular component; MF, molecular function. Kyoto Encyclopedia of Genes and Genomes enriched pathway analysis of differentially expressed miRNAs in the skeletal muscle tissue in the swimming group and model control group (B), huangqi group and model control group (D), and combine treatment group and model control group (F). Enrichment analysis was performed by Fisher's exact test. *P* < 0.05 was used as the threshold.**

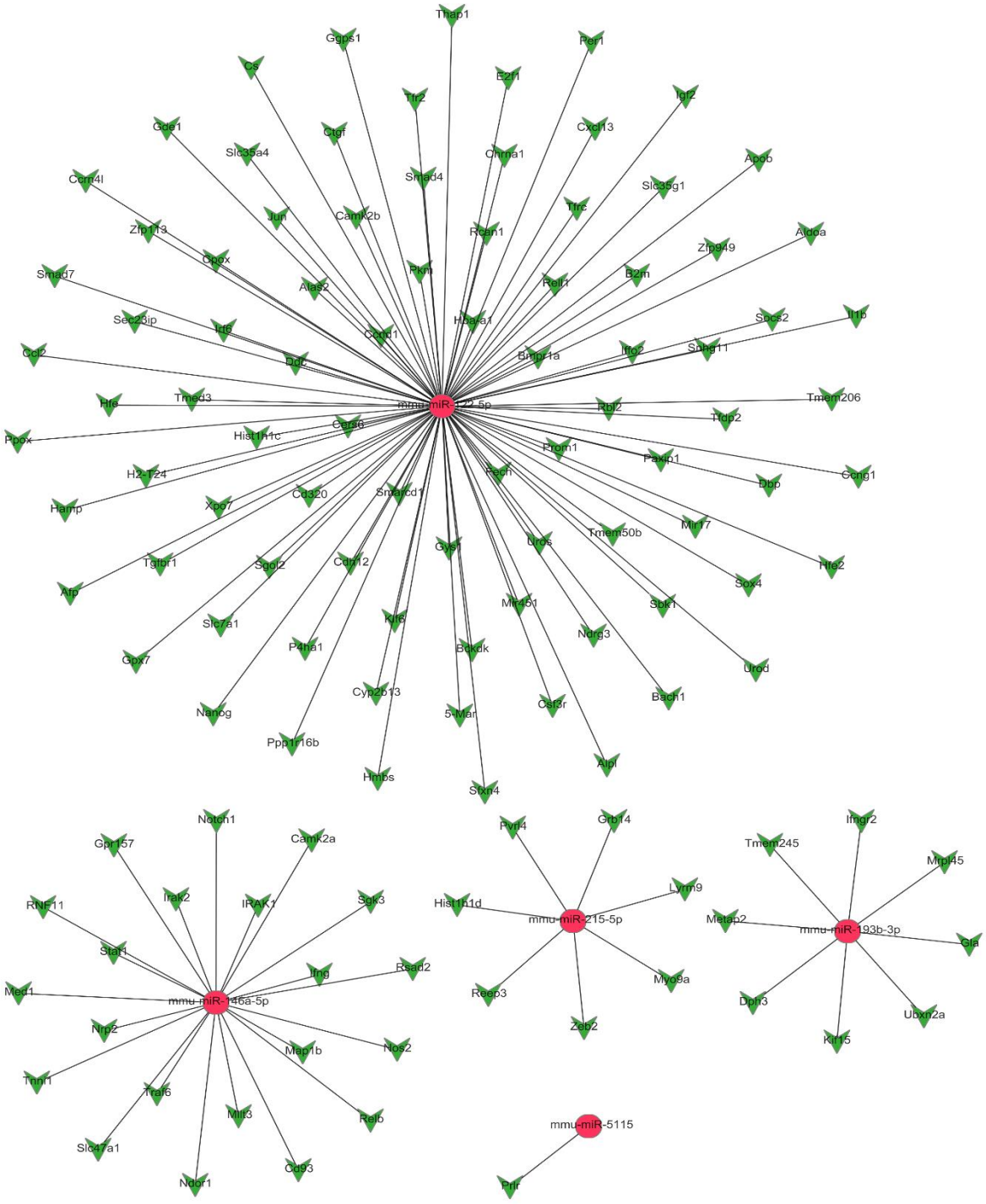

Figure 5. **Interaction network for the miRNAs and target genes among those groups. Red indicate differentially expressed miRNAs; green indicate target genes.**